Pico Protein Express Assay User Guide

For LabChip® GXII Touch

CE

P/N CLS140168 Rev. G
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Specifications

Assay Specifications

Table 1. Assay Specifications

Sizing Range	14 kDa - 200 kDa
Sizing Resolution[a]	± 10% difference in molecular weight
Sizing Accuracy[b]	± 20%
Assay Antibody Linear Concentration Range	50 pg/µL - 500 ng/µL (4 logs)
Antibody LOD in Original Sample	50 pg/µL
Assay Protein Linear Concentration Range	10 pg/µL - 100 ng/µL (4 logs)
Protein LOD in Original Sample	10 pg/µL
Sensitivity of % Purity	0.1% of the total protein
Chip Lifetime	HT Chip: 400 samples 24 Chip: 200 samples
Samples per Chip Prep	HT: up to 400 samples (four 96-well plates or one 384-well plate), or LT: up to 96 samples
Chip Preps per Reagent Kit	4 HT or LT chip preps

a. Resolution is defined as the height of the valley between two peaks to be no more than 50% of the maximum peak height. Actual separation performance can depend on the sample and application.

b. Labeling efficiencies will vary from protein to protein.

Sample Conditions

Table 2. Sample Conditions

Buffers, Salts and Additives	Prior to analysis, ensure your sample buffers meet the following requirements: • pH between 7.5 - 9 • Do not contain amines • Do not have a higher molar buffer concentration than the 1X labeling buffer, which is 0.1 M Sodium Bicarbonate, pH 8, 0.14% w/v LDS. Amines will interfere with the labeling of the protein. If the buffer your samples are in has a pH that is outside the above range, contains amines and/or has a molar concentration greater than the labeling buffer, we recommend performing a buffer exchange into the labeling buffer included in the kit.
Sample Protein Concentration	The optimal protein concentration range for labeling is 100 - 1000 ng/µL, although users may go as low as 50 pg/µL starting concentration for certain antibodies and 10 pg/µL starting concentration for proteins.
Standard Curve	PerkinElmer recommends users perform a standard curve analysis to accurately quantify a specific antibody or protein. Refer to Preparing a Protein Standard Curve on page 29.
Carryover Contamination	Users can prevent carryover contamination from high protein samples by always following a high protein sample (>2 ng/µL protein or >10 ng/µL Ab) with blank protein wells (buffer only wells).
Particulates	Sample plates should be spun down prior to analysis. All buffers should be filtered with a 0.22 µm cellulose acetate filter.
Salt Concentration	Total salt concentration in the final sample must not exceed 1M. Salt concentrations higher than 1M will interfere with chip fluidics. Salt introduced by the original sample will be diluted to varying degrees in the final sample depending on the protocol.

Storage Conditions

Chip Storage: Prior to use, store chips at 2 - 8°C. After use, store chips at room temperature for up to 30 days.

Reagent Storage: Store Lyophilized Labeling Dye (blue cap) at -20°C. Store all other reagents at 2 - 8°C. Store reconstituted dye in DMSO for up to one week at 2 - 8°C. Store reconstituted dye in DMSO, if not used within one week, at -20°C. The reconstituted dye in DMSO has demonstrated stability up to 2 years at -20°C.

CRITICAL:
The chip and all refrigerated reagents must equilibrate to room temperature (20 - 25°C) for at least 30 minutes before use. Remove the Lyophilized Labeling Dye from the padded shipping pack and allow to warm from -20°C to room temperature for 45 minutes, protected from light.

Reagent Kit Contents

Pico Protein Express Reagent Kit, P/N 760498

Table 3. Reagents

Reagent	Vial	Quantity
Pico Protein Express 5X Labeling Buffer	Clear	1 vial, 1.5 mL
Lyophilized Labeling Dye	Blue	5 vials
Pico Protein Express Sample Buffer	White	5 vials, 1.8 mL each
Pico Protein Express Gel Matrix	Red	2 vials, 1.8 mL each
Pico Protein Express Ladder	Yellow	1 vial, 0.08 mL
Pico Protein Express Lower Marker	Green	1 vial, 0.5 mL
Pico Protein Express Wash Buffer	Purple	4 vials, 1.8 mL each
Pico Protein Express Stop Buffer	Orange	1 vial, 1.3 mL
DMSO	Brown	1 vial, 1.0 mL

Table 4. Consumable Items

Item	Supplier and Catalog Number	Quantity
Detection Window Cleaning Cloth	VWR®, Cat. # 21912-046	1
Swab	ITW Texwipe®, Cat. # TX758B	3
Centrifuge Tubes, 2.0 mL	(Not sold separately)	5
Ladder Tubes, 0.2 mL	(Not sold separately)	10
Buffer Tubes, 0.75 mL	(Not sold separately)	10

Protein Express LabChips

Table 5. Protein Express LabChips

Item	Part Number
HT Protein Express LabChip (for use with LabChip GXII Touch HT)	760499
Protein Express LabChip (24) (for use with LabChip GXII Touch 24 or HT)	CLS138950

Safety and Usage

Safety Warnings and Precautions

CAUTION

We recommend that this product and components be handled only by those who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. As all chemicals should be considered potentially hazardous, it is advisable when handling chemical reagents to wear suitable protective clothing, such as laboratory overalls, safety glasses, and gloves. Care should be taken to avoid contact with skin or eyes. In case of contact with skin or eyes, wash immediately with water.

WARNING!

- *Wash Buffer and Sample Buffer contain LDS. Avoid inhalation and contact with skin and eyes.*
- *Protein Gel Matrix contains Methyl urea. Avoid contact with skin and eyes.*

Usage

The Pico Protein Express Assay is for use with the LabChip GXII Touch instrument. LabChip GXII Touch instruments are for research use only and not for use in diagnostic procedures.

Preparation Procedures

CRITICAL:

- *The chip and all refrigerated reagents must equilibrate to room temperature (20 - 25°C) for at least 30 minutes before use.*
- *Remove the Lyophilized Labeling Dye from the padded shipping pack and allow to warm from -20°C storage to room temperature (20 - 25°C) for 45 minutes, protected from light.*
- *The assay requires exact and consistent adherence to the protocol as shown in this guide, or results may be compromised by increased variability.*
- *Fresh Milli-Q® water should be obtained the day of the assay.*
- *Adherence to the full vortex time is important for assay performance.*

Additional Items Required

- 0.6 mL centrifuge tubes and/or 96-well plates for denaturing protein samples.
- Means for heating samples to 100°C — 96-well PCR instrument or heating block.

Note: *Avoid using non-stick lab consumables. They may induce unexpected or erratic assay results caused by surface treatments leaching into dye or gel components.*

- 18 megohm, 0.22-µm filtered water (Milli-Q® or equivalent).
- 70% isopropanol solution in DI water.
- Reducing agent: 1M DTT (dithiothreitol)
- Analyte Buffer (PBS, 20mM Potassium Acetate (KOAc)
- Alkylating agent (10M N-ethylmaleimide (NEM)) dissolved in DMSO (freshly prepared each day) for non-reduced protocols
- Alkylating agent (0.5M Iodoacetamide (IAM)) for alternate labeling protocol for sensitive antibodies

Preparing the Dye Solution

Note: *The dye is light sensitive. Do not expose dye to light for any length of time. Keep prepared dye in the dark.*

1. Centrifuge lyophilized dye (blue cap 🔵) in microcentrifuge for 1 minute at maximum speed, ensuring that the pellet is at the bottom of the tube.
2. Reconstitute dye to 200 µM by adding 120 µL of 100% DMSO (brown cap 🟤) to one vial of dye.
3. Just before labeling, dilute the 200 µM dye 5X with water (Milli-Q® or equivalent) to a concentration of 40 µM. Dilute only enough volume for immediate use. Diluted dye in water is not stable.
4. Store any unused 200 µM dye at 2 - 8ºC if used within one week, and at -20ºC for longer term storage.
5. Dispose of any unused 40 µM dye in the aqueous DMSO solution.

Preparing Diluted Stop Solution

1. Add 2 mL Pico Protein Express Sample Buffer (white cap ⚪) to 0.290 mL Pico Protein Express Stop Buffer (orange cap 🟠) and 10 mL of water (Milli-Q® or equivalent).
2. Label vial as “Diluted Stop Solution” and set aside until ready to use.

Protein Ladder Preparation

1. Add 4 µL Pico Protein Express Ladder (yellow cap) to 2 µL of Pico Protein Express 5X Labeling Buffer (clear cap) and 4 µL water (Milli-Q® or equivalent).
2. Vortex and centrifuge.
3. Denature at 75ºC for 5 minutes in an Eppendorf tube.
4. Vortex and spin down.
5. Add 5 µL of 40 µM reconstituted dye in water to 5 µL of denatured ladder.
6. Incubate for 60 minutes at room temperature (25ºC) in the dark.
7. Add 155 µL of diluted stop solution to the labeling reaction and vortex thoroughly. Spin down the reaction.
8. Store in the dark until ready to read samples on the instrument.
9. Transfer 120 µL of prepared ladder to the provided 0.2 mL Ladder Tube. Ensure there are no air bubbles in the Ladder Tube.
10. Insert the Ladder Tube into the ladder slot on the LabChip GXII Touch instrument.

Protein Sample Preparation

Note: *Samples can be prepared in either a 96-well or 384-well plate or in 0.6 mL microcentrifuge tubes (and subsequently pipetted into a plate).*

It is important to confirm that PerkinElmer's recommended protocol is compatible with your protein or antibody of interest. Proteins may vary in their content of lysines available for labeling, and their degree of sensitivity to fragmentation. PerkinElmer recommends titrating dye and protein concentrations and optimizing labeling times and temperatures for best results.

General Sample Preparation for Reduced/Denatured Proteins

Figure 1 on page 14 shows a sample preparation flowchart for reduced/denatured proteins.

1 **General Comments:** The optimal labeling pH is 7.5 - 9. The recommended optimal protein concentration range for labeling is 0.100 - 1 mg/mL. However, as an example, the general protocol uses 0.4 mg/mL of protein in 1X Labeling Buffer as a suggested starting point. Samples can be prepared in a microplate or in microcentrifuge tubes. If using a microplate, ensure that wells can fill to 110 µL (see step 9).

2 **Preparing the Pico Protein Express 5X Denaturing Labeling Buffer for Reduced Proteins**

   a Check to see if the Pico Protein Express 5X Labeling Buffer (clear cap ⚪) is cloudy. If so, dissolve precipitate by heating at 37ºC and vortexing occasionally until clear (1 - 2 minutes).

   b In an Eppendorf tube, add 34 µL of 1M DTT (user-provided) to 166 µL of Pico Protein Express 5X Labeling Buffer (clear cap ⚪).

   c Vortex and centrifuge tube.

3 **Denaturing and Labeling the Samples**

   a Dilute protein sample in prepared Pico Protein Express 5X Labeling Buffer/DTT from step 2 down to a 1x concentration of Labeling Buffer/DTT. For example, add 4 µL of sample protein at 0.4 mg/mL (range: 0.100 mg/mL to 1 mg/mL) to 1 µL of 5x Labeling Buffer/DTT.

Note: *It is only necessary to perform the last dilution in 5X Labeling Buffer/DTT. Initial dilutions may be made in either PBS or 20 mM KOAc.*

b Denature 5 µL of 0.4 mg/mL sample in 1X Labeling Buffer/DTT at 75°C for 5 minutes (see Figure 1 on page 14). Optimum denaturing conditions may vary by sample type.

c Add or dispense 5 µL of 40 µM dye to 5 µL of denatured sample.

d Seal plate and mix for 1 minute with shaker and spin at 1,000 xg for 1 minute. If the labeling reaction is in an Eppendorf tube, samples can be vortexed and centrifuged.

e Incubate at room temperature (25ºC) for 60 minutes in the dark. During incubation, prepare a second plate containing 105 µL of diluted stop solution.

f Shake and centrifuge plate or vortex and centrifuge sample in the Eppendorf tube.

g Transfer 5 µL of labeled sample to the second plate containing 105 µL of diluted stop solution.

h Seal and mix plate on shaker for 1 minute.

i Centrifuge plate (1,000 xg for 1 minute).

j Read on the GXII Touch using the Pico Protein Express assay.

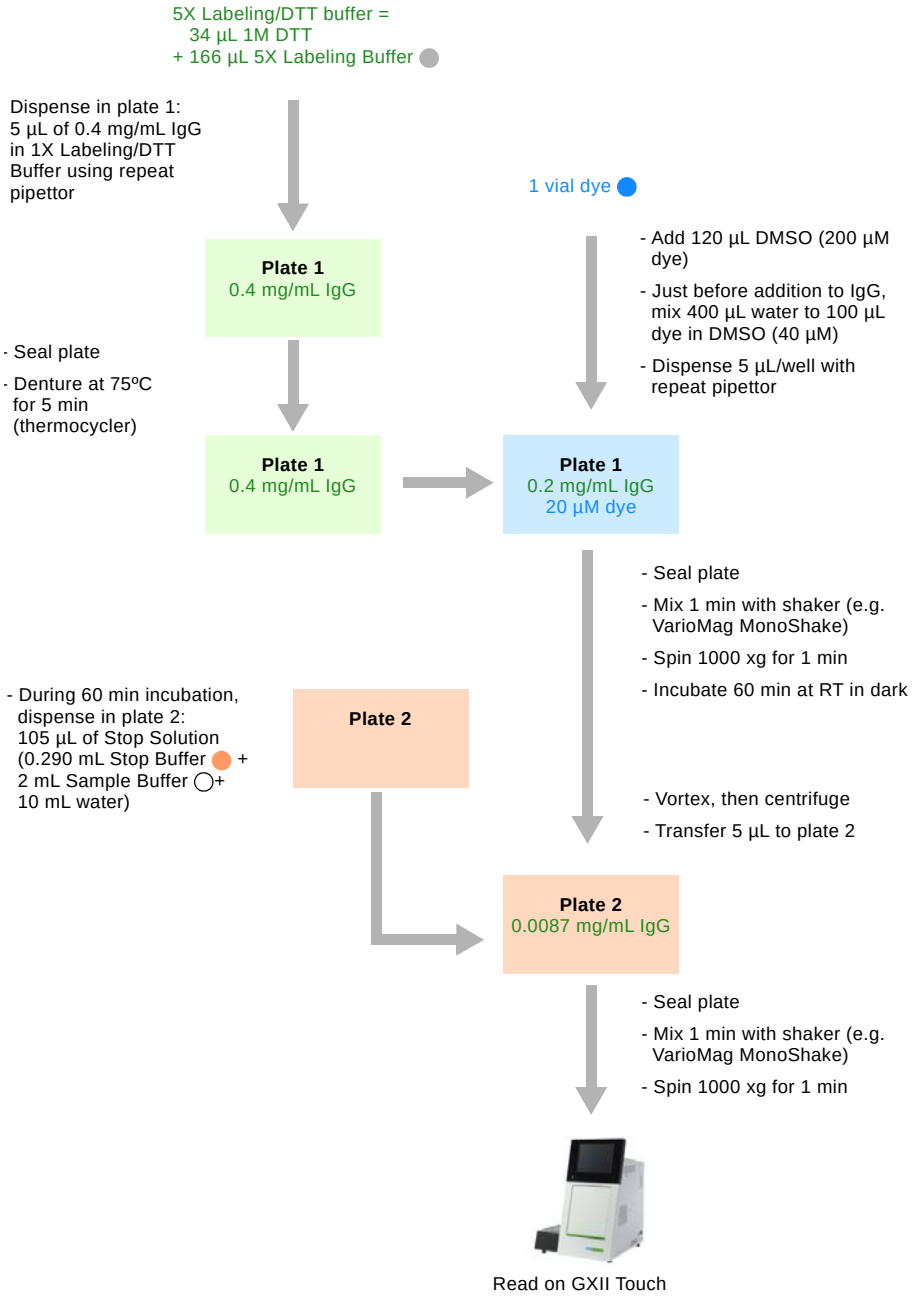

Figure 1. Flowchart for reduced proteins: Example sample preparation for starting concentration of 0.4 mg/mL

General Sample Preparation for Non-reduced Proteins

Figure 2 on page 17 shows a sample preparation flowchart for non-reduced proteins.

1 **General Comments:** The optimal labeling pH is 7.5 - 9. The recommended optimal protein concentration range for labeling is 0.100 - 1 mg/mL. However, as an example, the general protocol uses 0.4 mg/mL of protein in 1X Labeling Buffer as a suggested starting point. Samples can be prepared in a microplate or in microcentrifuge tubes. If using a microplate, ensure that wells can fill to 110 µL (see step 9).

2 **Preparing the Pico Protein Express 5X Labeling Buffer/NEM for Non-reduced Proteins**

a Check to see if the Pico Protein Express 5X Labeling Buffer (clear cap ⚪) is cloudy. If so, dissolve precipitate by heating at 37ºC and vortexing occasionally until clear (1 - 2 minutes).

b To prepare Pico Protein Express 5xNEM Labeling Buffer for non-reduced proteins, add 20 µL of 10 M NEM in DMSO (prepared fresh i.e. 0.125 g + 100 µL DMSO) to 14 µL water (Milli-Q® or equivalent) and 166 µL of Pico Protein Express 5x Labeling Buffer (clear cap ⚪). This is enough for 100 reactions.

Note: *Pico 5X Labeling Buffer/NEM should not be reused — scale preparation as necessary and dispose any unused portion at the end of the run.*

c Vortex and centrifuge tube.Set aside until needed.

3 **Dilute Protein Sample in Prepared Pico 5X Labeling Buffer/NEM**

a The protein sample must be diluted in Labeling Buffer/NEM down to a 1X concentration of labeling buffer/NEM. For example, add 4 µL of sample protein at 0.4 mg/mL (range: 0.100 mg/mL to 1 mg/mL) to 1 µL of 5x NEM Labeling Buffer.

Note: *It is only necessary to perform the last dilution in 5X Labeling Buffer/*NEM*. Initial dilutions may be made in either PBS or 20 mM KOAc.*

b Denature 5 µL of 0.4 mg/mL sample in 1X Labeling Buffer/NEM at 75°C for 5 minutes (see Figure 2 on page 17). Optimum denaturing conditions may vary by sample type.

c Add or dispense 5 µL of 40 µM dye to 5 µL of denatured sample.

d Seal plate and mix for 1 minute with shaker and spin at 1,000 xg for 1 minute. If the labeling reaction is in an Eppendorf tube, samples can be vortexed and centrifuged.

e Incubate at room temperature (25ºC) for 60 minutes in the dark. During incubation, prepare a second plate containing 105 µL of diluted stop solution.

f Shake and centrifuge plate or vortex and centrifuge sample in the Eppendorf tube.

g Transfer 5 µL of labeled sample to second plate containing 105 µL of diluted stop solution.

h Seal and mix plate on shaker for 1 minute.

i Centrifuge plate.

j Read on the GXII Touch using the Pico Protein Express assay.

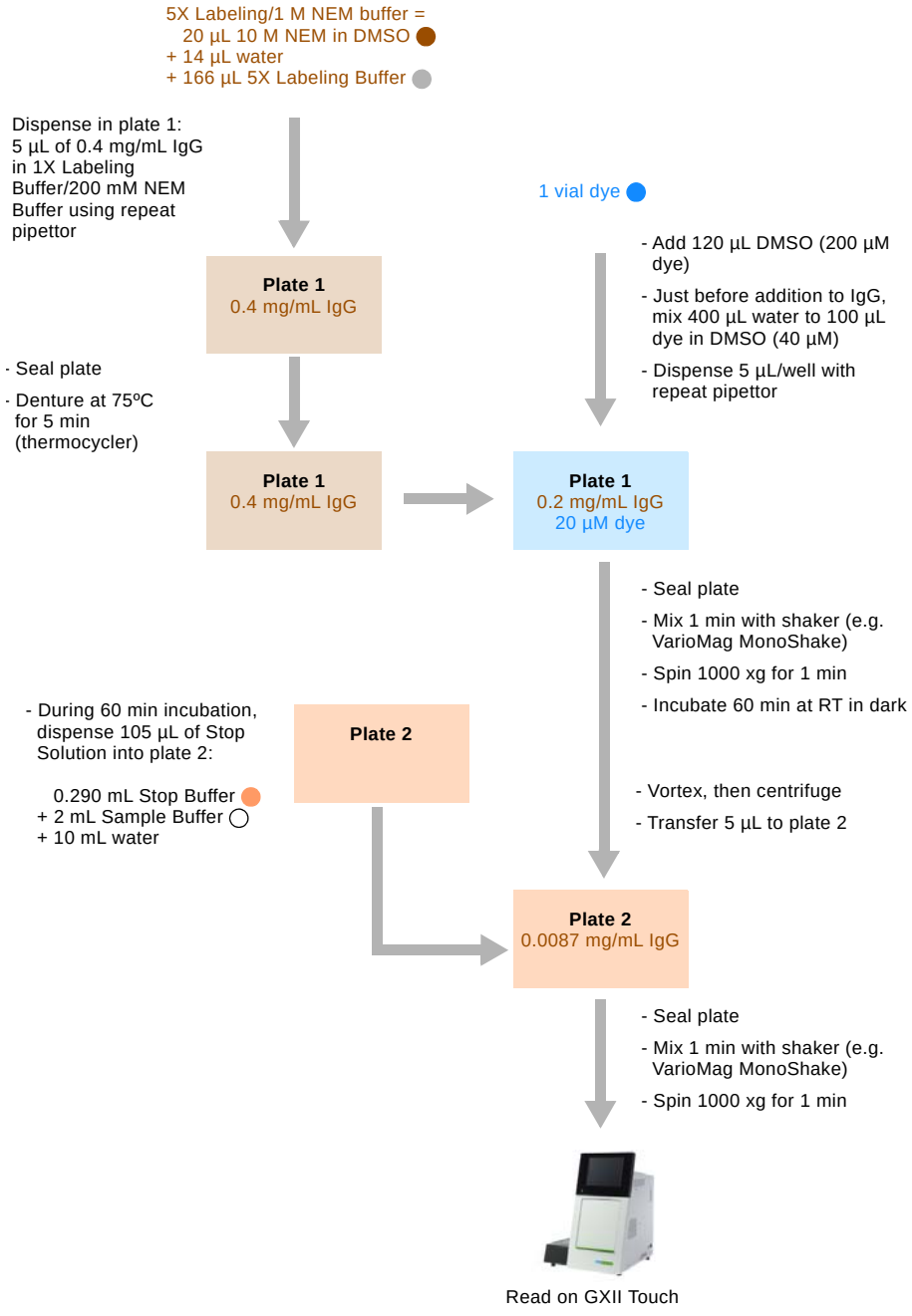

Figure 2. Flowchart for non-reduced proteins: Example sample preparation for starting concentration of 0.4 mg/mL

Preparing the Buffer Tube

1 Add 750 µL of Pico Protein Express Wash Buffer (purple cap ●) to the 0.75 mL Buffer Tube provided with the reagent kit. Ensure there are no air bubbles in the Buffer Tube.

2 Insert the Buffer Tube into the buffer slot on the LabChip GXII Touch instrument.

Note: *Replace the Buffer Tube with a freshly prepared tube every 8 hours when the chip and instrument are in use.*

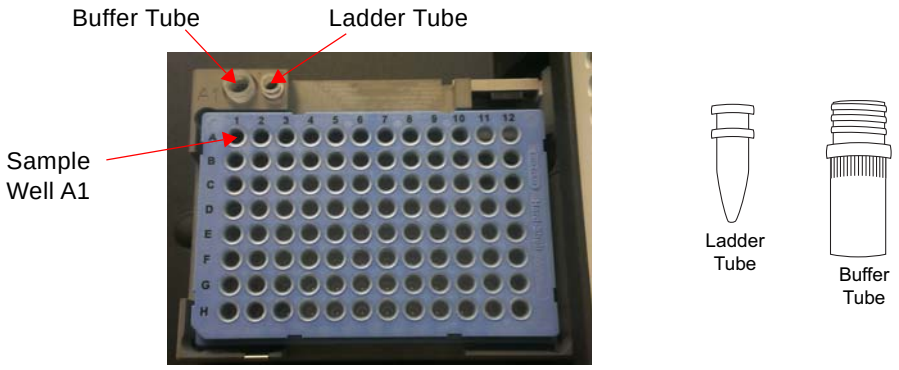

Figure 3. Locations of the Buffer Tube and Ladder Tube in the GXII Touch instrument

Preparing the Chip

1 Allow the chip to equilibrate to room temperature (20 - 25°C) for at least 30 minutes before use.

2 Use a pipette tip attached to a vacuum line to thoroughly aspirate all fluid from the chip wells (see Figure 4). For more details on how to set up a vacuum line see page 53.

3 Rinse and completely aspirate each active chip well (1, 2, 3, 4, 7, 8, 9, and 10) twice with water (Milli-Q® or equivalent). Do not allow active wells to remain dry.

4 If any water spilled onto the top or bottom of the chip surfaces during rinsing, aspirate using the vacuum line. DO NOT run the tip over the central region of the detection window. Use the provided Detection Window Cleaning Cloth to clean the chip detection window.

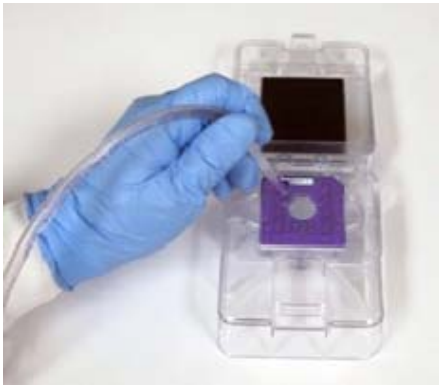

Figure 4. Using a vacuum to aspirate the chip wells is more effective than using a pipette. See page 53 for more details

5 Using a reverse pipetting technique, add 75 µL Protein Gel Matrix solution (red cap ●) to chip wells 2, 3, 7, 8, and 9, and 120 µL to well 10 as shown in Figure 5.

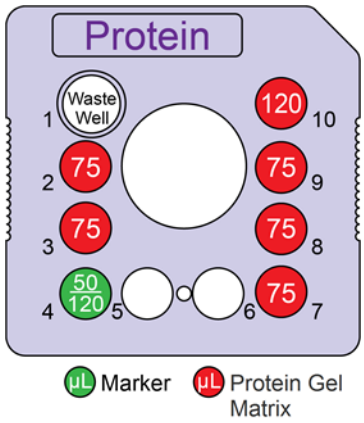

Figure 5. Reagent placement

6 If the chip will be used to analyze multiple 96-well plates or will be in use for up to 8 hours, add 120 µL of Pico Protein Express Lower Marker (green cap ●) to chip well 4. If the chip will only be used to analyze one 96-well plate or a partial plate and then stored for future use, the marker volume can be reduced to 50 µL. Make sure the marker volume is pipetted accurately. If there is not enough marker in chip well 4, the marker will deplete and will not be added to subsequent samples on-chip. Data collected without marker peaks cannot be analyzed by the software.

7 Make sure the rims of the chip wells are clean and dry.

8 **IMPORTANT:** Ensure chip well 1 (waste well) is empty before placing the chip into the instrument.

Inserting a Chip into the LabChip GXII Touch Instrument

1 Check that the sample plate, Buffer Tube, and Ladder Tube are placed on the instrument properly.

2 Remove the chip from the chip storage container and inspect the chip window. Clean BOTH sides of the chip window with the PerkinElmer-supplied Detection Window Cleaning cloth dampened with a 70% isopropanol solution in DI water.

3 Touch the *Unload Chip* button on the *Home* screen.

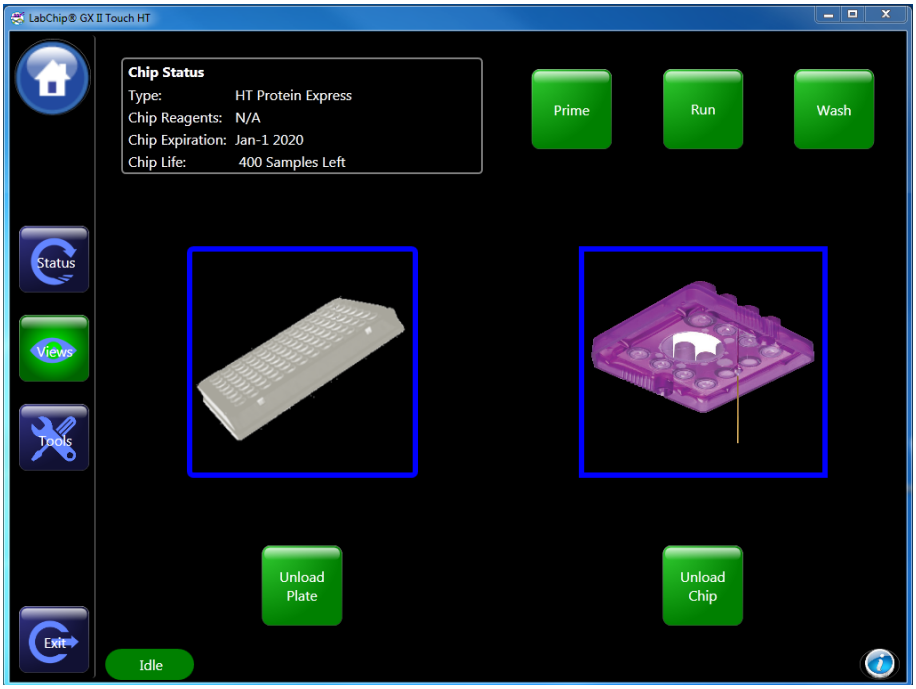

Figure 6. *Home* screen

4 Insert the chip into the LabChip GXII Touch instrument (Figure 7) and close the chip door securely.

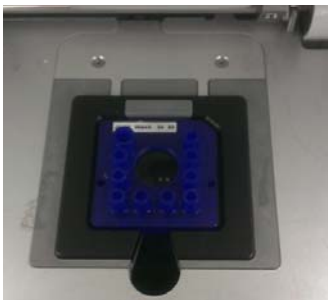

Figure 7. Chip in the LabChip GXII Touch instrument

5 Touch the *Load Plate* button on the *Home* screen (Figure 6) to retract the sample plate and move the sipper to the Buffer Tube.

Note: *Do not keep the chip door open for any length of time. Dye is sensitive to light and can be photobleached.*

Running the Assay

Note: *Chips can be primed independently from running assays. Touch the Prime button on the Home screen (Figure 6). Select the desired assay from the Assay drop-down list (see Figure 9). Touch the Prime button on the Chip Priming screen. Make sure the Buffer Tube is placed on the instrument.*

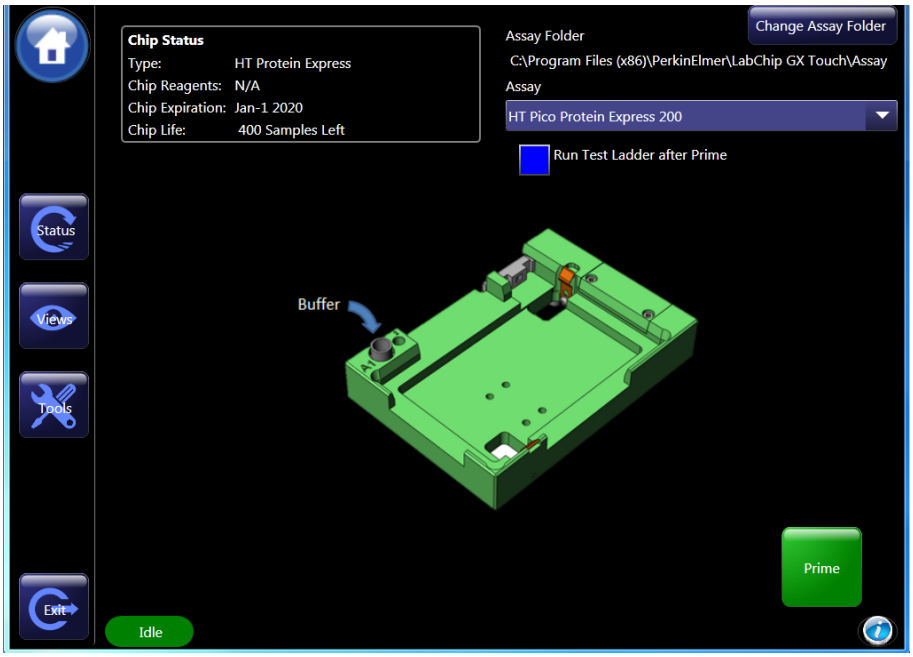

Figure 8. Chip Priming screen

1 Touch the *Run* button on the *Home* screen (see Figure 6 on page 20).

2 Select the appropriate assay type (see Figure 9), plate name, well pattern, and whether to read wells in columns or rows. Select number of times each well is sampled under *Adv. Settings* (Figure 10). Touch the green arrow.

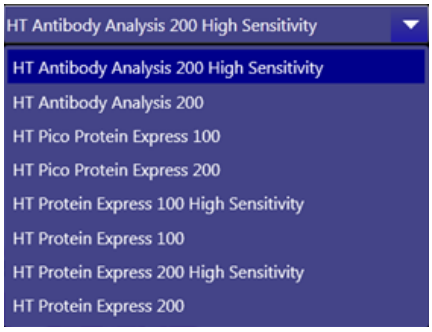

Figure 9. The *Assay* List

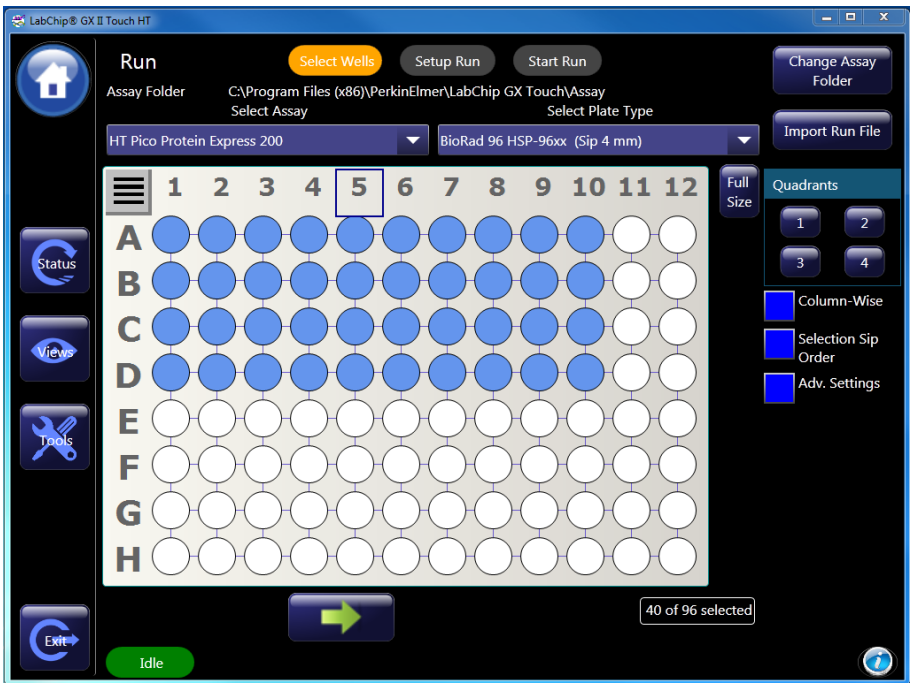

Figure 10. Selecting wells

For Pico Protein Express assays, appropriate assay types are:

- **Pico Protein Express 100:** For sizing of proteins in 14 - 100 kDa range
- **Pico Protein Express 200:** For sizing of proteins in 14 - 200 kDa range

3 In the *Setup Run* tab, select the operator name, the option to read barcode, the destination of the file, the inclusion of sample names, expected peaks, and excluded peaks and the filename convention. Select *Auto Export* to export results tables automatically (Figure 11). Touch the green arrow.

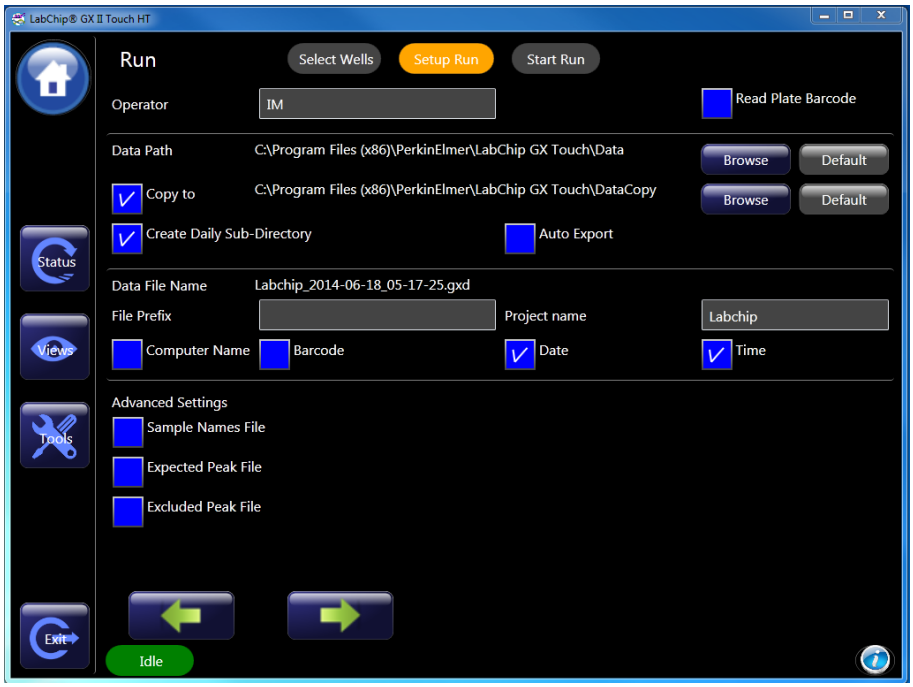

Figure 11. Run setup screen

4 Touch *Start* to begin the run.

Figure 12. Starting a run

Cleaning and Storing the Chip

After use, the chip must be cleaned and stored in the chip container.

1. Place the chip into the chip storage container. The sipper should be submerged in the fluid reservoir.
2. Remove the reagents from each well of the chip using vacuum.
3. Rinse and completely aspirate each active well (1, 2, 3, 4, 7, 8, 9, and 10) twice with water (Milli-Q® or equivalent).
4. Add 120 µL of water (Milli-Q® or equivalent) to the active wells.
5. Cover the wells with Parafilm® to prevent evaporation and store the chip at room temperature (20 - 25°C) until next use. Allowing chip wells to dry may lead to changes in chip performance. Use to the total lifetime within 30 days of analyzing the first sample. See Assay Specifications on page 4 for Chip Lifetime.

Chip Cartridge Cleaning

1. **Daily**
   - **a** Inspect the inside of the chip cartridge and O-rings for debris.
   - **b** Use the provided lint-free swab dampened with water (Milli-Q® or equivalent) to clean the O-rings using a circular motion. If the O-rings stick to the chip or a pressure leak is detected, perform the more extensive monthly cleaning procedure.
2. **Monthly**
   - **a** To reduce pressure leaks at the chip interface, clean the O-rings frequently. Remove the O-rings from the top plate of the chip interface on the LabChip GXII Touch instrument. Soak O-rings in water (Milli-Q® or equivalent) for a few minutes. Clean the O-ring faces by rubbing between two fingers. Wear gloves.
   - **b** To reduce the occurrence of current leaks, clean the chip interface frequently. Clean the top plate of the chip interface using the provided lint free swab dampened with water (Milli-Q® or equivalent).
   - **c** Allow the O-rings and chip interface to air dry. Reinsert the O-rings into the chip cartridge.

Alternate Preparation Procedures

Labeling Reduced Protein of Unknown or Very Low Protein Concentration

If the protein concentration is unknown, we recommend assuming that the concentration is low. This low throughput protocol increases the dye starting concentration to 200 µM dye without further dilution in water (Milli-Q® or equivalent). For very low protein concentrations, users can increase dye concentration to as much as 600 µM in DMSO. However, users should keep DMSO contribution into the labeling reaction to 10%. If the peak heights are found to be saturating, further dilutions of the protein are necessary before labeling.

Note: *Users should substitute the general ladder protocol with the associated ladder labeling protocol that follows in* *Labeling Ladder for Unknown or Very Low Protein Concentration on page 26**.*

Recommended Protocol

1. Check to see if the Pico Protein Express 5X Labeling Buffer (clear cap ⚪) is cloudy. If so, dissolve precipitate by heating at 37ºC until clear (1 - 2 minutes).
2. Add 17 µL of 1M DTT (user-provided) to 83 µL of Pico 5X Labeling Buffer.
3. Vortex and centrifuge sample.
4. In an Eppendorf tube, add 1 µL Pico 5X Labeling Buffer/DTT (prepared in step 2) directly to 4 µL of protein sample.
5. Denature samples at 75°C for 5 minutes. Optimum denaturing conditions may vary by sample type.
6. Add 1 µL of 200 µM (or higher) dye to 4 µL of denatured sample from step 1.
7. Incubate at room temperature (25ºC) for 60 minutes in the dark.
8. Vortex and centrifuge sample.
9. In an Eppendorf tube, add 5 µL of labeled sample to 105 µL of diluted stop solution.
10. Vortex and centrifuge sample.
11. Read samples on GXII Touch instrument.

Labeling Ladder for Unknown or Very Low Protein Concentration

This ladder labeling protocol should be used in association with the prior protocol for samples of unknown concentration or very low protein concentration.

Recommended Protocol

1. In an Eppendorf tube, denature 4 µL of Pico Protein Express ladder (yellow cap) at 75°C for 5 minutes.
2. Centrifuge tube.
3. Add 1 µL of 200 µM Dye to tube.
4. Incubate at room temperature (25ºC) for 60 minutes in the dark.
5. Vortex and centrifuge sample.
6. Add 5 µL of labeled sample to 325 µL of diluted Stop Buffer.
7. Vortex and centrifuge sample.
8. Transfer 120 µL of prepared ladder to the provided 0.2 mL Ladder Tube. Ensure there are no bubbles in the Ladder Tube.
9. Insert the Ladder Tube into ladder slot on the LabChip GXII Touch instrument.

Labeling Non-reduced Samples for Sensitive Antibodies

If the recommended sample preparation protocol for non-reduced proteins results in higher than expected level of fragmentation, PerkinElmer recommends the following substitution. Denaturing prior to labeling may induce fragmentation for certain sensitive antibodies. This protocol labels antibodies in the absence of any detergent as a first step and denatures at the end of the protocol.

Recommended Protocol

1. Substitute 5X Labeling Buffer with 0.5 M Sodium Bicarbonate, pH 8.0.
2. Dilute antibody sample down to a working concentration in 0.1 M Sodium Bicarbonate.
3. Combine 5 µL of 40 µM dye with 5 µL of antibody.
4. Incubate at room temperature (25ºC) for 60 minutes in the dark.
5. Add 1 µL of Stop Buffer (orange cap ●)
6. Vortex and centrifuge sample.
7. To 5 µL of labeled antibody, add 18 µL of Sample Buffer/IAM (50 µL Sample Buffer (white cap ◯)+ 5 µL 0.5 M IAM)
8. Denature sample at 75ºC for 5 minutes.
9. Add 77 µL water (Milli-Q® or equivalent).
10. Vortex and centrifuge sample.

Labeling Reduced Antibodies for HC/LC Analysis

To quantify HC/LC ratios for antibodies, PerkinElmer recommends using the protocol below for accurate HC/LC analysis for obtaining best results. Labeling efficiency is maximized for both the light and heavy chains of typical antibodies. In this protocol, the dye is kept undiluted at 200 µM in DMSO and the protein concentration is increased to 1 mg/mL.

Note: *Users should substitute the general ladder protocol with the associated* *Labeling Protein Ladder for HC/LC Analysis on page 28**.*

Recommended Protocol

1. Prepare 20 µL of protein sample in 1X Labeling Buffer at a protein concentration of 1 mg/mL.
2. Denature samples at 75°C for 5 minutes. Optimum denaturing conditions may vary by sample type.
3. Add 1 µL of 200 µM Dye to 4 µL of denatured protein at 1 mg/mL.
4. Incubate at room temperature (25ºC) for 60 minutes in the dark.
5. Vortex and centrifuge sample.
6. Add 435 µL of diluted stop solution to the 5 µL of labeled sample.
7. Vortex and centrifuge sample.
8. Transfer 45 µL of labeled protein to a 96-well plate.
9. Read on GXII Touch instrument.

Labeling Protein Ladder for HC/LC Analysis

If running the protocol for reduced antibodies, users must also run its associated protein ladder labeling procedure for obtaining best results. This ladder labeling procedure uses the same dye concentration as the reduced antibody protocol for HC/LC analysis but maintains the required ladder dilution factor for quantification.

Recommended Protocol

1. Denature 20 µL of protein ladder at 75°C for 5 minutes.
2. Add 1 µL of 200 µM Dye to 4 µL of Pico Protein Express ladder (yellow cap).
3. Incubate at room temperature (25ºC) for 60 minutes in the dark.
4. Vortex and centrifuge sample.
5. Add 325 µL of diluted Stop Solution to the 5 µL labeled ladder.
6. Vortex and centrifuge sample.
7. Transfer 120 µL of prepared ladder to the provided 0.2 mL Ladder Tube. Ensure there are no bubbles in the Ladder Tube.
8. Insert the Ladder Tube into ladder slot on the LabChip GXII Touch instrument.

Preparing a Protein Standard Curve

1. Starting with 250 ng/µL protein concentration or higher, dilute protein down, using 1:2-fold dilutions in an appropriate analyte buffer, until you reach 7.8 ng/µL.

Table 6. Dilution series.

Dilution	Concentration
1	250 ng/µL
2	125 ng/µL
3	62.5 ng/µL
4	31.25 ng/µL
5	15.6 ng/µL
6	7.8 ng/µL

2. Prepare each protein sample dilution as you would normally to find the optimal working concentration for your protein of interest. Users may label their protein at a single high concentration and perform the serial dilution. However, the results obtained at any lower dilution may not represent the actual labeling efficiency if a user actually started at that dilution. Alternatively, you can serially dilute the protein and label each dilution. Although this standard curve will contain more noise, it will be a better representation of actual labeling efficiency.

3. We recommend that at very high and very low protein concentrations, dye to protein ratios be increased above 1:15 for maximum labeling efficiency. At low protein concentrations, the kinetics may require longer incubation times which may be undesirable to the user. Higher dye concentrations will mitigate this issue. At very high protein concentrations, more dye molecules are required to label all available sites on the protein. A general rule of thumb is to use 20 µM dye at reaction or 1:15 protein/dye ratio, whichever is lower at any given protein concentration. It is also recommended that the concentration of DMSO (dye) included in each reaction be kept constant since varying DMSO concentration might influence labeling efficiency. It is recommended that DMSO be no more than 10% of the labeling reaction.

4. To quantify the sample peak concentration based on the protein standard curve, refer to the LabChip GX Touch software manual for analyzing protein standard curves.

Results

Pico Protein Express Ladder Data

The electropherogram of a typical Protein Express ladder is shown in Figure 13. Peaks to the right of the lower marker and system peaks in order of increasing migration time correspond to proteins of increasing size i.e., 15.9 kDa, 20.4 kDa, 28.9 kDa, 48.4 kDa, 68.4 kDa and 119.2 kDa respectively.

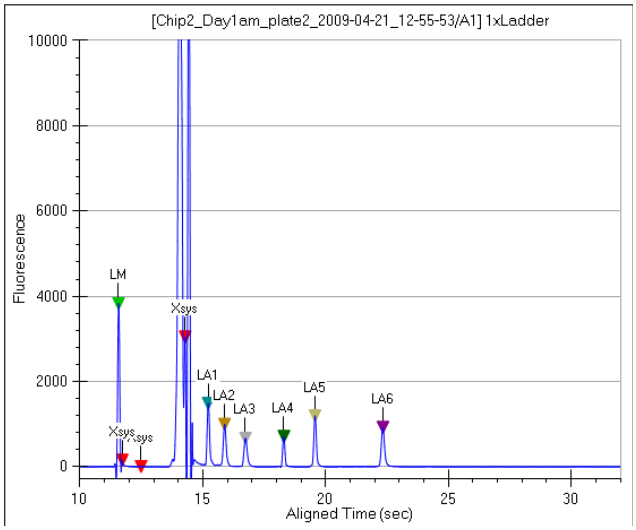

Figure 13. Pico Protein Express ladder electropherogram

Reduced/Denatured Protein Data

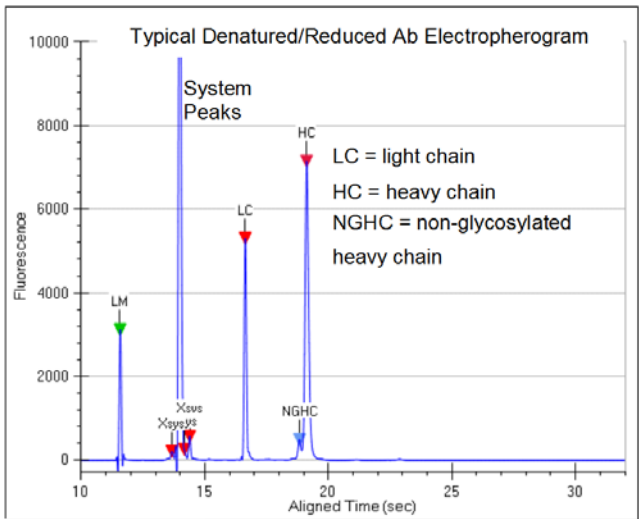

Figure 14. Pico Protein Express ladder electropherogram for reduced protein

Non-reduced Protein Data

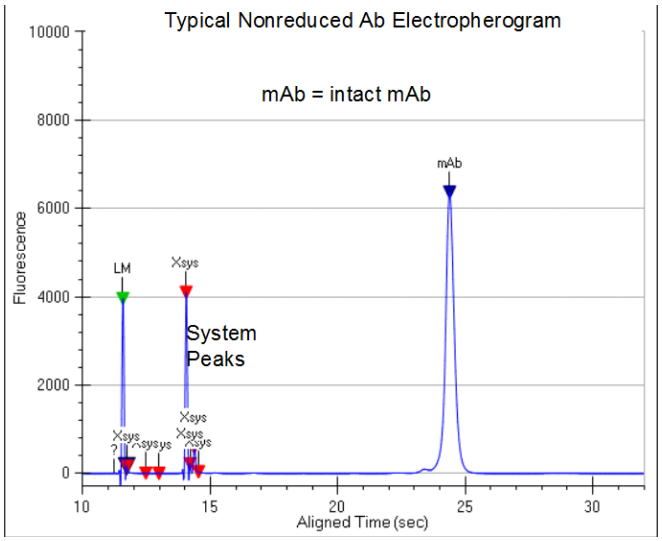

Figure 15. Pico Protein Express ladder electropherogram for non-reduced protein

High-protein Data with 120 µM Dye at Reaction

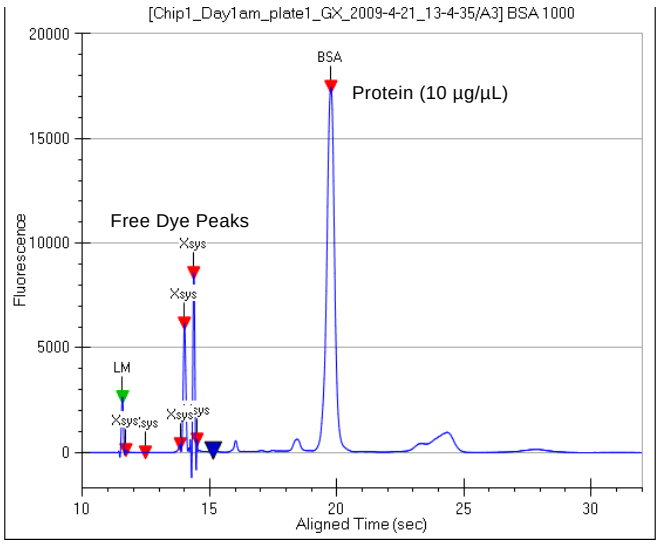

Figure 16. Pico Protein Express electropherogram for high-protein sample (10 µg/mL) with 120 µM dye

Low-protein Data with 120 µM Dye at Reaction

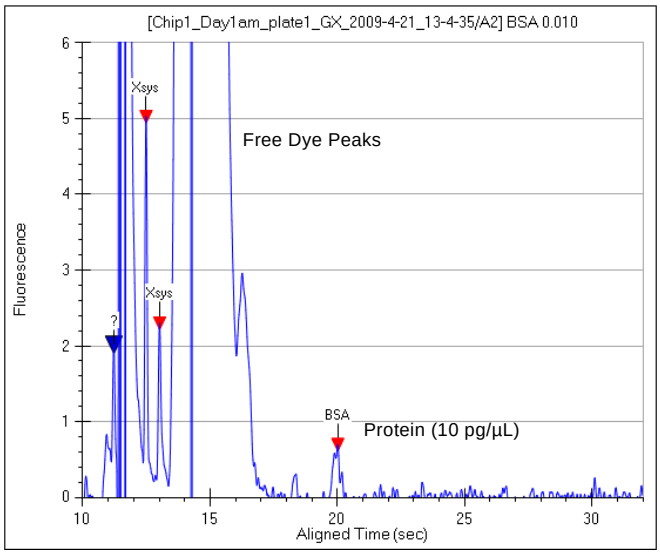

Figure 17. Pico Protein Express electropherogram for low-protein sample (10 pg/mL) with 120 µM dye

Troubleshooting

Note: *Some of the data examples shown in this section were generated with assays other than the assay described in this user guide.*

Symptom: No ladder or sample peaks but marker peaks detected.

Note: *The lower marker peak height will most likely be greater than normal height.*

Possible causes:

1 Air bubble in sipper introduced during chip priming.

What to do:

1 Reprime the chip. See Repriming Chips on page 49 for instructions on how to reprime the chip.

Symptom: Missing sample, ladder *and* marker peaks.

Possible causes:

1 Clog in sipper or marker channel of chip.

What to do:

1 Reprime the chip. See Repriming Chips on page 49 for instructions on how to reprime the chip.

Symptom: Ladder detected but no sample peaks.

Possible causes:

1 The sipper is not reaching the sample due to low sample volume in the well of the plate.

2 If the missing sample peaks occurred only in a few wells of the plate, check those wells for air bubbles.

3 The sipper is not reaching the sample due to an incorrect capillary height setting or incorrect plate definition.

4 If the plate has been uncovered for some time, sample evaporation might have occurred.

5 Debris from the sample or sample prep is clogging the sipper.

What to do:

1 Add more sample to the well.

2 Manually insert a larger volume pipette tip (~100 µL) into the sample well and dislodge the bubble. Rerun these sample wells.

3 Check the plate definitions.

4 Check the sample wells, especially around the edge of the plate where evaporation is fastest, and make a fresh plate if volumes are low.

5 If you suspect there may be debris in your samples, spin the sample plate down in a centrifuge (e.g., 3000 rpm for 5 minutes). Unclog the sipper by repriming the chip. See Repriming Chips on page 49 for instructions on how to reprime the chip.

Symptom: No ladder peaks but sample peaks and marker peaks are present.

Possible causes:

1 Low or no ladder volume in the Ladder Tube.

What to do:

1 Add more ladder to the Ladder Tube and restart the run. Recommended standard ladder volume is 120 µL (minimum volume is 100 µL).

Symptom: No marker peaks but sample peaks are present.

Possible causes:

1 No marker added to chip well 4.

2 If there is marker solution in chip well 4, the problem may be due to a marker channel clog.

What to do:

1 This may be due to not filling marker well or chip remaining idle on instrument for extended period of time. Add or replenish the marker solution in the chip using the following procedure:

- Touch the *Unload Chip* button on the Home screen to open the chip door.
- Return the chip to the chip container ensuring the sipper is immersed in fluid.
- Thoroughly aspirate all fluid from chip well 4 using a vacuum line.
- Rinse and completely aspirate chip well 4 twice with water (Milli-Q® or equivalent).
- Add Marker Solution (green cap ●) to chip well 4.

- Reinsert the chip back into the instrument.
- Restart the run.

2 Perform a marker channel unclogging procedure by repriming the chip. See Repriming Chips on page 49 for instructions on how to reprime the chip.

Symptom: Ladder traces show up in the lanes following the ladders (delayed sip).

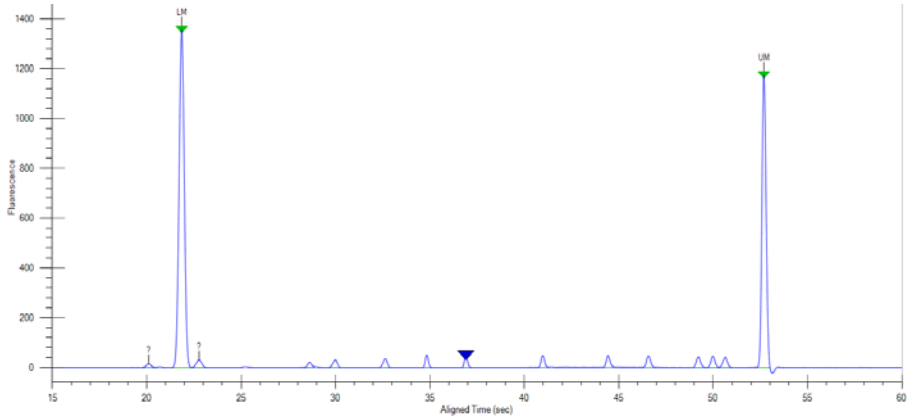

Figure 18. Small ladder peaks in sample well caused by delayed sip

Possible causes:

1 Separation channel overloaded with sample.

2 Partial clog in the separation channel.

What to do:

1 Lower the starting sample concentration.

2 Reprime the chip. See Repriming Chips on page 49 for instructions on how to reprime the chip.

Symptom: Unexpected sharp peaks.

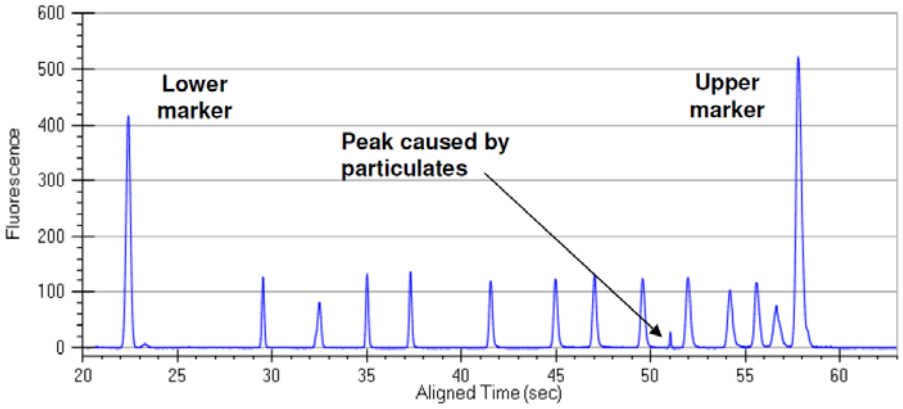

Figure 19. Unexpected sharp peak

Possible causes:

- Dust or other particulates introduced through sample or reagents.

What to do:

1 Do one or all of the following:

- Replace the 18 megohm, 0.22-µm filtered water (Milli-Q® or equivalent) water used for chip preparation.
- Replace the buffer used for sample and reagent preparation.
- Use a 0.22-micron filter for all water and buffers used for chip, sample, and reagent preparation.
- Spin down sample plate to pellet any particulates.

Symptom: Humps in several electropherograms which do not correspond to sample data.

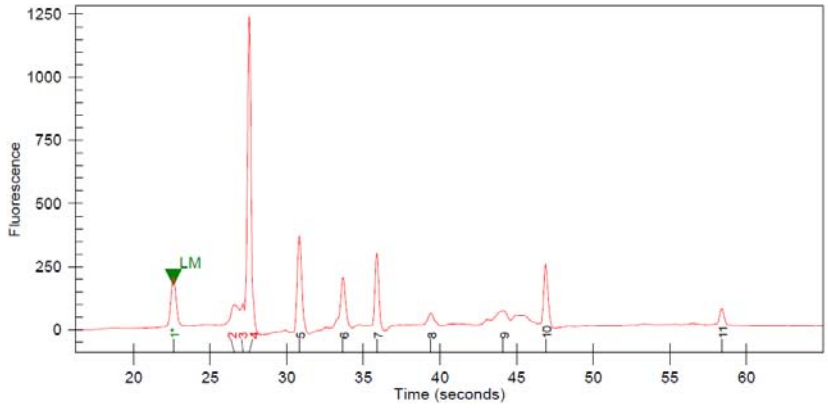

Figure 20. Humps in several electropherograms

Possible causes:

1. Electrode 7 is dirty and has contaminated the gel in well 7.
2. High concentrations of detergent in the sample buffer can sometimes cause humps in the electropherogram.

What to do:

1. Before restarting the run, clean electrode 7. Remove the chip and follow the electrode cleaning procedure. We recommend using the provided swab and isopropanol to manually clean electrode 7.
2. Lower the detergent concentration in the sample.

Symptom: Peaks migrating much faster or slower than expected.

Note: *Some migration time variance between chips or within a plate is considered normal chip performance. All chips are QC tested at PerkinElmer prior to shipment.*

Possible causes:

1. Particulates from the samples may be clogging the separation channel (this will slow down migration).
2. Gel was not primed properly into the chip.

What to do:

1. Wash and reprime the chip with fresh Protein Gel Matrix solution. See Repriming Chips on page 49 for instructions on how to wash and reprime the chip.
2. If fast or slow migration is observed repeatedly on a new chip, contact technical support to arrange return of the chip to PerkinElmer. Please send a data file showing the failure along with the return request.
3. Minimize the loading of particulates in the sample by performing a centrifuge spin of the sample plate (e.g. 3000 rpm for 5 minutes) and/or ensuring the Sip 4 mm plate type is selected in the Select Wells screen before starting a new run. The debris may be flushed out of the chip by washing and re-priming the chip. See Repriming Chips on page 49 for instructions on how to wash and reprime the chip.
4. Check the O-rings on the top surface of the chip interface and clean if necessary.

Frequently Asked Questions

Protein Detection

1 How does the software determine protein concentration?

Protein samples contain a single lower marker. Alignment to a single marker does not provide enough constraints to align large proteins, so data is aligned to two ladders, one sipped just before the sample wells and another sipped just after the sample wells. Samples are scaled so the sample's lower marker is nearly aligned with both ladder lower markers. The scaling is weighted by the proximity in sip time to each ladder. The sample sipped closest to the primary ladder is scaled to align more closely to the primary ladder and the sample sipped just before the bracket ladder is scaled to align most closely to the bracket ladder. Then each sample is shifted in time so the sample's lower marker aligns exactly with the primary ladder lower marker. After alignment, the size of the protein producing each peak is calculated from the aligned peak time using a log (size) versus 1/Time fit to the primary ladder peaks of known size and measured migration time. This fit to the ladder peak data can be viewed by selecting Standard Curve from the Analysis Menu.

To determine sample peak concentration, the peak areas are first corrected to compensate for the fact that the fluorescence intensities are sampled at a constant time interval so slower moving proteins spend more time under the detector than fast moving proteins. The peak concentration is then calculated using the ladder peak areas and concentration for the ladder supplied in the Assay Analysis window. The concentration is adjusted for the differing dilution ratios of sample and ladder.

2 How can I use the information from the protein standard curve to adjust the protein concentrations?

To quantify the sample peak concentration based on the protein standard curve, the new standard must be added into each sample well at a known concentration. The analysis settings provide a Sample Peak Quantitation option using the peak area and concentration of the User Standard instead of the ladder concentrations.

Users may label their protein at a single high concentration and perform the serial dilution. However, the results obtained at any lower dilution may not represent the actual labeling efficiency if a user actually started at that dilution.

Alternatively, users can serially dilute their protein and label each dilution. Although this standard curve will contain more noise, it will be a better representation of actual labeling efficiency.

We recommend that at very high or very low protein concentrations, dye to protein ratios be increased above 1:15 for maximum labeling efficiency. At low protein concentrations, the kinetics may require longer incubation times, which may be undesirable to the user. Higher dye concentrations will mitigate this issue. At very high protein concentrations, more dye molecules are required to label all available sites on the protein.

A general rule of thumb is to use 20 µM dye at reaction or 1:15 protein/dye ratio, whichever results in the greater amount of dye at any given protein concentration. It is also recommended that the concentration of DMSO included in each reaction be kept constant since this might also influence labeling efficiency. It is recommended that the amount of DMSO be no higher than 10% of the labeling reaction.

3 What is the optimal protein concentration for labeling?

The recommended optimal protein concentration range for labeling is 100 - 1000 ng/uL, although lower protein concentrations can also work. It is important to note that the protein to dye ratio should remain constant if protein concentration is to vary.

4 What is the optimal protein to dye ratio?

The optimal protein to dye ratio will vary from protein to protein. However, PerkinElmer has found that the range of optimal ratios falls typically falls between 1:6 to 1:15. It is recommended that users find the optimal ratio for their protein of interest. When labeling very high or very low protein concentrations, users may have to increase the amount of dye to protein used. As a quick rule of thumb: use 20 µM of dye in the labeling reaction or a 1:15 ratio, whichever is greater. See question 2 for related information.

5 Can one manually override the quantification and use known protein standard values?

Yes. LabChip GX Touch software supports a user-defined standard curve to improve concentration estimates.

6 Do I have to worry about carryover on the Protein Express LabChip chips?

Unless the Ab starting concentration is greater than 10 ng/µL or 2 ng/µL for proteins, carryover should not be a problem. For proteins above these concentrations, users should follow high-protein wells with a no-protein well (protein buffer only) before the next sample well is read on the plate.

7 What is the lowest level of contamination that the Pico Protein Express Reagent Kit can detect?

The kit is able to detect down to 0.1% contamination of protein. This is based on a protein starting concentration of 400 ng/uL.

8 Can the Pico Protein Express Reagent Kit detect aggregates of antibodies?

Yes. Covalent aggregates can be detected. If users wish to determine the level of covalent aggregates held together by disulfide bonds, they will have to run both non-reduced and reduced protocols. Generally, higher protein concentrations will aid in the detection of aggregates since populations tend to represent only a small percentage of total sample being analyzed.

9 Can the Pico Protein Express Reagent Kit detect antibody glycosylation?

Yes, the kit can distinguish between glycosylated and non-glycosylated heavy chain, and determine glycan occupancy.

Kit Specifications

1 What are the storage conditions for the various Pico Protein Express Reagent Kit components?

Store Lyophilized Labeling Dye at -20ºC. Store all other reagents at 2 - 8ºC. Store reconstituted dye in DMSO for up to one week at 2 - 8ºC. Store reconstituted dye in DMSO, if not used within one week, at -20ºC.

Store unused chips at 2 - 8ºC. After first use, store chips at room temperature (20 - 25°C) for up to 30 days. Use chips within 30 days of first use.

2 How stable is the reconstituted dye and how can I extend the life of the dye?

The reconstituted dye in DMSO has demonstrated stability up to 2 years at -20ºC. Reconstituted dye in DMSO can be kept at 2 - 8ºC for shorter periods as it is being used in the short term. Once the dye is reconstituted with water, it is not stable. Dye reconstituted in water should not be kept for periods longer than one hour.

3 My protein seems to require higher than normal dye to protein ratios. I am running out of dye before the rest of the reagents in the Pico Protein Express Reagent Kit are used up. Can I purchase the dye separately?

There is currently no plan to sell the dye separately.

4 Why must the 10 M NEM be made fresh each day?

NEM reconstituted in DMSO is not considered stable and must be made fresh each day. Non-reduced antibodies may fragment when using old 10 M NEM.

5 What is the shelf life of the Pico Protein Express Reagent Kit and its components?

Six months.

6 Can the Pico Protein Express Reagent Kit be used in a Code of Federal Regulation (CFR) environment?

Yes. PerkinElmer enables running the LabChip GX Touch/GXII Touch suite of instruments in regulated environments. The GxP Software package provides compatibility with 21 CFR Part 11 requirements. Users are responsible for establishing policies and standard operating procedures that complement the capabilities provided by the software in order to ensure complete compliance to the rule.

7 What additional materials are required to run the assay?

Users are asked to provide water (Milli-Q® or equivalent), 70% isopropanol, 1 M DTT, analyte buffer and 10 M NEM in DMSO. It is preferable that users use their own analyte buffer, which should abide by the recommended pH conditions.

8 How stable is NEM once it has been reconstituted with the labeling buffer?

N-ethylmaleimide is unstable at room temperature after reconstitution in DMSO, ethanol or water and must be used within an hour. Unused 10 M NEM should be discarded at the end of each day. However, aliquots of 10 M NEM in DMSO can be kept frozen at -20ºC for single use only.

9 What proteins does PerkinElmer use to validate the linear concentration range of its kit?

PerkinElmer uses a monoclonal antibody and non-acetylated Bovine Serum Albumin to verify the kit specifications, Bovine Albumin (Fraction V powder, fatty acid free, low endotoxin) from Sigma, Cat. # A-8806. Labeling efficiencies will vary from protein to protein, depending on buffer compatibilities, pH dependences and the number of lysine residues available for labeling.

10 Why doesn't PerkinElmer have specifications on the accuracy of this kit's protein quantification?

The quantification of proteins in the Pico Protein Express Reagent Kit is based on comparison against a standard protein ladder. There is no guarantee that the labeling of PerkinElmer protein ladder will reflect the efficiency of labeling for any particular protein a user might be investigating. Generally, it is best to prepare a standard protein ladder for better quantification.

Assay Preparation

1 How long does sample prep take?

Generally, sample prep should take no more than 2 hours before the plate is ready to be analyzed on the GXII Touch instrument. Of course, this time will vary depending on the number of samples and the complexity of the experiment.

2 What protein buffers are compatible with this assay?

It is important to note that there are pH recommendations for buffers to be used with the Pico Protein Express Reagent Kit. If unsure if a particular buffer is compatible with labeling, one can always perform a buffer exchange with the buffers provided in the kit.

3 What are the guidelines for buffer exchange?

PerkinElmer recommends that users use centrifuge type columns and perform three exchanges into the buffer of choice. Each exchange should be in a 1:10 dilution of the original buffer. The original buffer should not be a higher molarity than the final buffer.

4 How long does it take to run 12 or 96 samples on the GXII Touch?

After the chip and samples have been prepared, it takes approximately 30 minutes to run 12 samples and approximately 80 minutes to run 96 samples. The processing time is less than 50 seconds per well.

5 Can I process both denatured/reduced antibodies *and* non-reduced antibodies on the 96-well plate?

Yes. The general protocol can be used to process both reduced and non-reduced samples on the same plate. See question 1 above.

6 My protein seems to be fragmenting during the non-reduced protocol. How can I better protect sensitive antibodies or chimeric antibodies?

Sensitive antibodies or proteins may require a more gentle labeling buffer and/or procedure. If your protein appears to be sensitive, follow the procedure given in Labeling Non-reduced Samples for Sensitive Antibodies on page 27.

7 The lyophilized dye pellet seems smaller than usual when I reconstituted it. Should I continue using it?

If you observe the reconstituted dye is either clear or noticeably lighter in coloration, contact PerkinElmer Technical Support.

8 My protein sample has an unknown concentration. Which protocol should I use?

It is best to prepare a protein standard curve to quantify an unknown. However, if the sample is limited, then we would recommend the "Labeling Ladder for Unknown or Very Low Protein Concentration" protocol on page 26. If the signal saturates, the sample will need to be diluted to analyze it.

9 If I am using the Pico Protein Express Reagent Kit for the first time, how do I confirm it's working properly?

Users may confirm kit performance by labeling commercially available non-acetylated bovine serum albumin. See question 9 in Kit Specifications on page 40.

10 Can I use TCEP (Tris(2-Carboxyethyl) phosphine) instead of DTT to reduce my protein?

PerkinElmer does not recommend use of TCEP to reduce proteins.

11 What steps should I take to optimize the Pico Protein Express Reagent Kit for my protein(s) of interest? How many proteins can I optimize in one kit?

Users can follow the general protocol for reduced or non-reduced proteins as a first step. If the results match well against orthogonal methods, no further optimizations are necessary.

If labeling efficiencies seem low under reduced conditions for antibodies, users should try to increase dye to protein ratio, protein concentration or length of labeling. As a quick first step, users may want to try the Labeling Reduced Antibodies for HC/LC analysis. This method incorporates higher concentrations of protein and dye for optimal labeling. However, individual results may vary and further optimizations may be required.

If protein samples seem to be fragmenting in non-reduced conditions, try lowering the 75°C incubation time from 5 minutes or decreasing the temperature. Alternatively, try the protocol detailed in Labeling Non-reduced Samples for Sensitive Antibodies on page 27. However, this non-high throughput method deviates from the general protocol and cannot be used in conjunction with the reduced protocol on the same plate. If you suspect that the protein is under-labeled, try increasing the dye to protein ratio, protein concentration or length of labeling to more than an hour.

Depending on the stability of the protein, the number of available sites for labeling and other characteristics of a particular protein, the number of optimizations will vary. Typically, users should be able to optimize for several proteins with one kit.

12 What's the difference between using the general protocol for reduced protein (page 12) and the labeling reduced antibodies for HC/LC analysis protocol (page 27).

The general protocol uses a lower concentration of protein and dye than the HC/LC specific protocol. The dye in the general protocol is reduced to 40 µM by the addition of water (Milli-Q® or equivalent). This dye dilution step maintains the protein to dye ratio at 1:15 when the protein starting input is 0.4 mg/mL. The general protocol also avoids adding dye in volumes less than 5 µL. It also minimizes dye usage by diluting the dye but users should keep in mind that the dye should be used in a timely manner to reduced dye hydrolysis.

The HC/LC specific protocol does not have a dilution step and uses dye at 200 µM in DMSO. Although the protein concentration has been increased, the protein to dye ratio is the same as in the general protocol. This protocol will decrease the reactions a user can perform with one kit because the amount of dye used is higher. However, this protocol should increase the labeling efficiency of the protein.

13 Can I use non-stick Eppendorf tubes for the sample preparation procedure?

Yes, non-stick Eppendorf tubes are preferable when protein samples are at a very low concentration. However, PerkinElmer recommends that users avoid silanized Eppendorf tubes which might affect assay performance.

Assay Principles

1 Can users substitute another dye for the Pico Protein Express Reagent Kit labeling dye?

PerkinElmer does not recommend substitution of the labeling dye with other dyes which may have decreased efficiencies and might be incompatible with its detection optics.

2 How can I improve protein labeling efficiency?

PerkinElmer recommends that users optimize labeling efficiency by evaluating different dye to protein ratios, protein concentration, length of labeling and possibly temperature. Proteins may label differently depending on the number of available lysine residues. Some proteins may only require a short period of time before reaching maximum labeling at a given temperature while others may require longer times, greater amounts of dye or different temperatures. See question 2 in *Protein Detection on page 38* *for further discussion.*

LabChip Kit Essential Practices

To ensure proper assay performance, follow the important handling practices described below. Failure to observe these guidelines may void the LabChip Kit product warranty.[1]

Note: *It is important to keep particulates out of the chip wells, channels, and capillary. Many of the following guidelines are designed to keep the chips particulate-free.*

For assay and instrument troubleshooting, refer to the LabChip GX Touch Software Help file or call PerkinElmer Technical Support at 1-800-762-4000.

General

- Allow the chip, sample plate, and all refrigerated reagents to equilibrate to room temperature for at least 30 minutes before use.
- Remove the Lyophilized Labeling Dye from the padded shipping pack and allowed to warm from -20°C to room temperature for 45 minutes, protected from light.
- Clean the O-rings in the chip interface weekly and the electrodes daily. Refer to the Instrument Users Guide Maintenance and Service section for procedures.
- Avoid use of powdered gloves. Use only non-powdered gloves when handling chips, reagents, sample plates, and when cleaning the instrument electrodes and electrode block.
- Calibrate laboratory pipettes regularly to ensure proper reagent dispensing.
- Only the PerkinElmer-supplied Detection Window Cleaning cloth can be used on the chip to clean the detection window.
- Water used for chip preparation procedures must be 18 megohm, 0.22-µm filtered water (Milli-Q® or equivalent).
- Using the “Reverse Pipetting Technique” will help avoid introducing bubbles into the chip when pipetting the gel.

1. PerkinElmer warrants that the LabChip Kit meets specification at the time of shipment, and is free from defects in material and workmanship. LabChip Kits are warranted for 60 days from the date of shipment. All claims under this warranty must be made within thirty days of the discovery of the defect.

Reverse Pipetting Technique

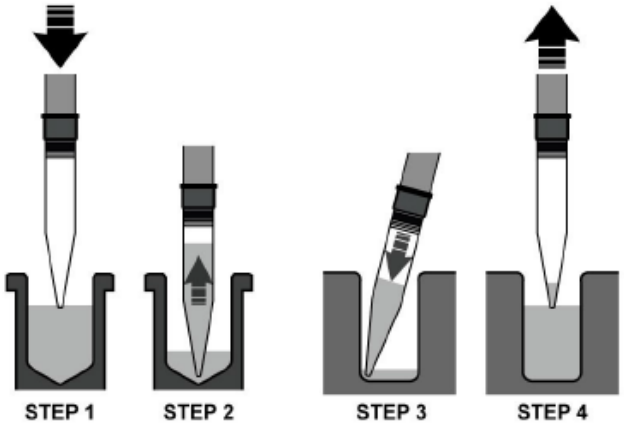

Figure 21. Reverse pipetting

1. Depress the pipette plunger to the second stop.
2. Aspirate the selected volume plus an excess amount from the tube.
3. Dispense the selected volume into the corner of the well by depressing plunger to the first stop.
4. Withdraw the pipette from the well.

Reagents

- Store reagents as specified in Storage Conditions on page 6.
- Dye reconstituted with water is not stable and should be used within one hour.
- For optimal performance, use one reagent kit per chip. The Pico Protein Express Reagent Kit contains the reagents to run four 96-well plates or four chip preparations, whichever comes first.

Chips

Repriming Chips

1. Touch the *Unload Chip* button on the *Home* screen to open the instrument door. Place the chip into the instrument.
2. Close the chip door securely and choose the corresponding assay.
3. Touch the *Prime* button on the *Home* screen to reprime the chip.

Washing Chips

Important Note: *Wash chips only with water (*Milli-Q® *or equivalent). Use of any other reagents (including Wash Buffer) is likely to cause even more artifacts in subsequent data.*

Notes: *Some protein samples may have components which produce data with extra peaks, spikes or other artifacts. When these artifacts are present, washing chips on the LabChip GXII Touch immediately before the next use can often restore data quality.*

Chips should only be washed on the LabChip GXII Touch immediately before they are prepared with fresh reagents and primed on the instrument. Chips should not be washed and left with water in the chip channels for any extended period of time.

*For most protein samples, the only chip cleaning protocol that is required is to rinse and aspirate the active wells twice with water (*Milli-Q® *or equivalent), and store the chip with 120 µL of water in each active well.*

To wash the chip:

1. Thoroughly aspirate all fluid from the chip wells using a vacuum line.

2 Rinse and completely aspirate each active well (1, 2, 3, 4, 7, 8, 9, and 10) twice with water (Milli-Q® or equivalent). Do not allow active wells to remain dry.

3 Add 120 µL of water (Milli-Q® or equivalent) to each active well (1, 2, 3, 4, 7, 8, 9, and 10).

4 Touch the *Unload Chip* button on the *Home* screen and place the chip into the instrument.

5 Close the chip door securely.

6 Transfer 750 µL of water (Milli-Q® or equivalent) to the Buffer Tube. Install into the instrument.

7 Touch the *Wash* button on the Home screen. Then touch the *Wash* button on the *Wash* screen (Figure 22).

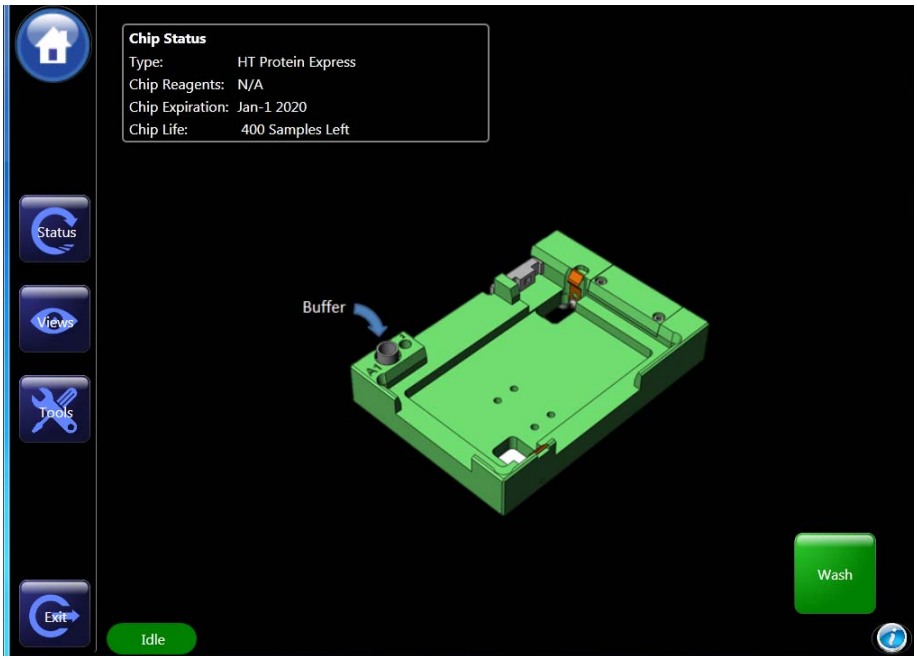

Figure 22. Wash screen

8 After completion of the wash cycle, open the chip cartridge and return the chip to the chip container ensuring the sipper is immersed in fluid.

9 Thoroughly aspirate all fluid from the chip wells using a vacuum line.

10 Replace fluid in the wells with freshly made reagents as described in Preparing the Chip on page 18. Do not let wells remain dry.

11 Transfer 750 µL of Wash Buffer (purple cap ●) into a clean Buffer Tube. Install into the instrument.

12 Install the Ladder Tube, sample plate and chip into the instrument and run the assay.

If air bubbles are not dislodged after a reprime, apply a vacuum to the sipper. Perform this by filling all active wells with 100 µL water (Milli-Q® or equivalent). Then suction the sipper with a vacuum line, as shown in Figure 23, until droplets of fluid flow out from the sipper. When suctioning the sipper, be careful not to bend or break the sipper. To facilitate this, cut the end of the pipette tip attached to the vacuum line to widen the mouth.

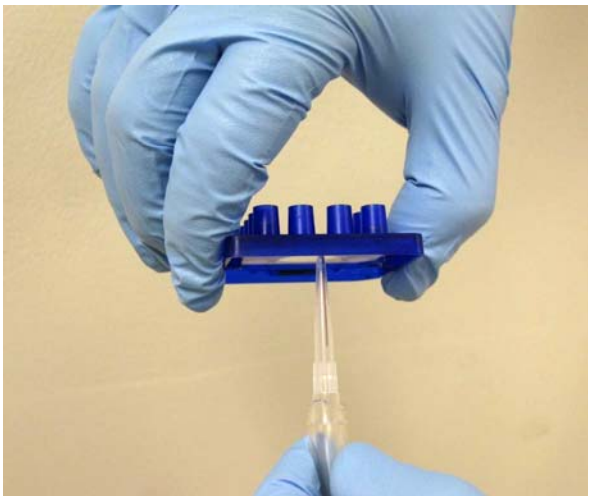

Figure 23. Removing an air bubble or clog by suctioning the sipper with a vacuum line

Other Considerations:

- Store chips as specified in Storage Conditions on page 6.
- After first use, store chips at room temperature and use within 30 days.
- Do not allow the liquid in the chip container to freeze, as this may lead to poor chip performance. Do not submerge the chip in any solution.
- The entire chip surface must be thoroughly dry before use.
- The sipper must be kept immersed in fluid at all times and should not be exposed to an open environment for long periods of time.
- Use care in chip handling to prevent sipper damage. Damage to the sipper can result in inconsistent sampling.

- Avoid exposing the chips to dust by keeping them in a closed environment such as in the chip container or in the instrument before and after chip preparation.
- Chips can be prepared and left in the instrument for extended periods of time so that samples can be run as needed throughout the day. PerkinElmer recommends the chip be re-prepared after it has been idle for 8 hours, but the chip can be used continually over an 8-hour work day as long as the maximum recommended idle time of 8 hours and total chip lifetime number of samples are not exceeded.

Samples

- Prepared sample plates should be free of gas bubbles and particulate debris, both of which may inhibit sipper flow.
- Spin down sample plates containing gas bubbles and/or particulate debris at 3000 rpm (1250 rcf) prior to analysis.
- Up to four 96-well plates (384 samples) can be run with a single chip preparation when running the GXII Touch HT instrument. Up to 48 samples can be run with a single chip preparation when running the GXII Touch 24 instrument.

Chip Well Aspiration Using a Vacuum

Aspirating with a pipette can leave used reagents in the chip wells. For this reason, PerkinElmer recommends vacuuming the wells instead. This can be accomplished by attaching a permanent pipette tip to a house vacuum line with trap (Figure 24). To avoid contamination, use a new disposable pipette tip over the permanent tip for each chip aspirated (Figure 25).

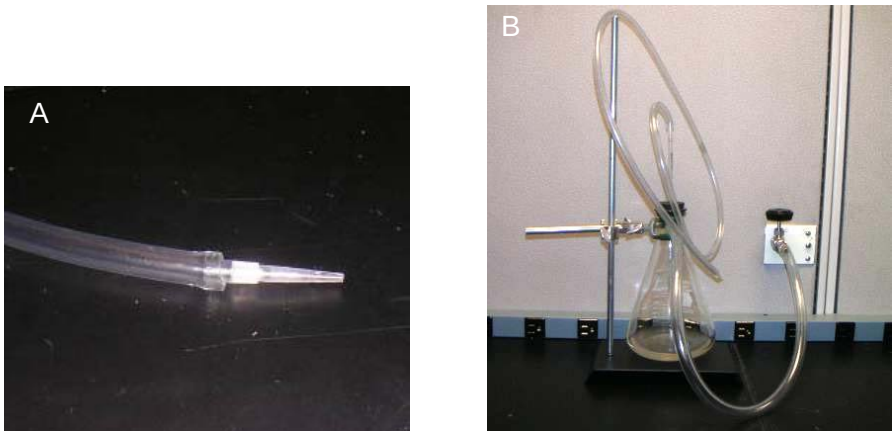

Figure 24. A: Permanent pipette tip attached to a house vacuum line; B: vacuum line with trap

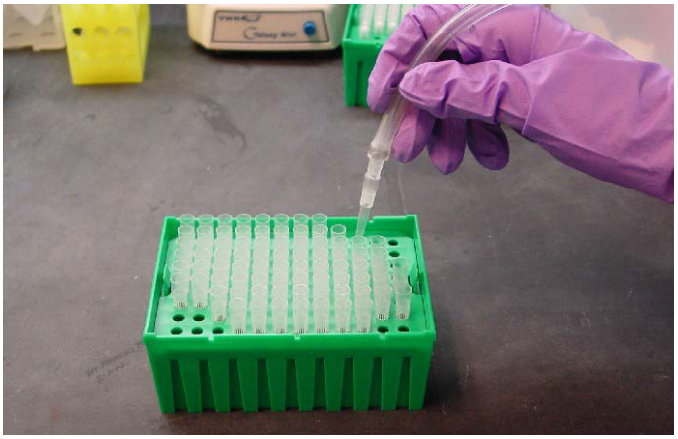

Figure 25. Replacing the disposable pipette tip

Reordering Information

Table 7. Reordering Information

Product	Part Number
Pico Protein Express Reagent Kit	760498
HT Protein Express LabChip, GXII Touch HT	760499
4-Pack HT Protein Express LabChip, GXII Touch HT	760528
Protein Express LabChip (24), GXII Touch 24 or HT	CLS138950
Pico Protein Express Sample Buffer, 25 mL bottle	760414
Detection Window Cleaning Cloth	VWR®, Cat. # 21912-046
Swab	ITW Texwipe®, Cat. # TX758B

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Email: dxsupportamericas@perkinelmer.com
Internet: www.perkinelmer.com

For additional assay and instrument troubleshooting, refer to the LabChip GX Touch Software Help file.

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