Complete Sample Protocol for PMA-Induced ERK Activation in Suspension Cell Lines

LI-COR Biosciences

Abstract

Developed for:

Aerius,
Odyssey® Classic,
Odyssey CLx, and
Odyssey Sa
Infrared Imaging Systems

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Guidelines

Required Reagents

LI-COR Reagents

- IRDye® 800CW Goat anti-Rabbit Secondary Antibody (LI-COR P/N 925-32210 or 926-32210)
- IRDye 680RD Goat anti-Mouse Secondary Antibody (LI-COR P/N 925-68070 or 926-68070)
- Odyssey® Blocking Buffer (LI-COR P/N 927-40000 or 927-50000)
- Large Western Incubation Box (LI-COR P/N 929-97301)

Additional Reagents

- 1X PBS wash buffer
- Tissue culture reagents (RPMI 1640, fetal bovine serum, etc.)
- Clear or black 96-well or 384-well microplates (See IX. Experimental Considerations)
- Jurkat cells (ATCC®, P/N TIB-152TM)
- THP-1 monocytes (ATCC, P/N TIB-202TM)
- K-562 lymphocytes (ATCC, P/N CCL-243TM)
- Concentrated Prefer (5X) (Anatech LTD, P/N 411)
- TO-PRO®-3 (Molecular Probes, P/N T-3605)
- PMA (phorbol 12-myristate 13-acetate) (Sigma®, P/N P1585)
- DMSO (dimethyl sulfoxide) (Sigma, P/N D8418)
- ERK rabbit antibody (Santa Cruz, P/N sc-94)
- pERK mouse antibody (Cell Signaling Technology, P/N 9106)

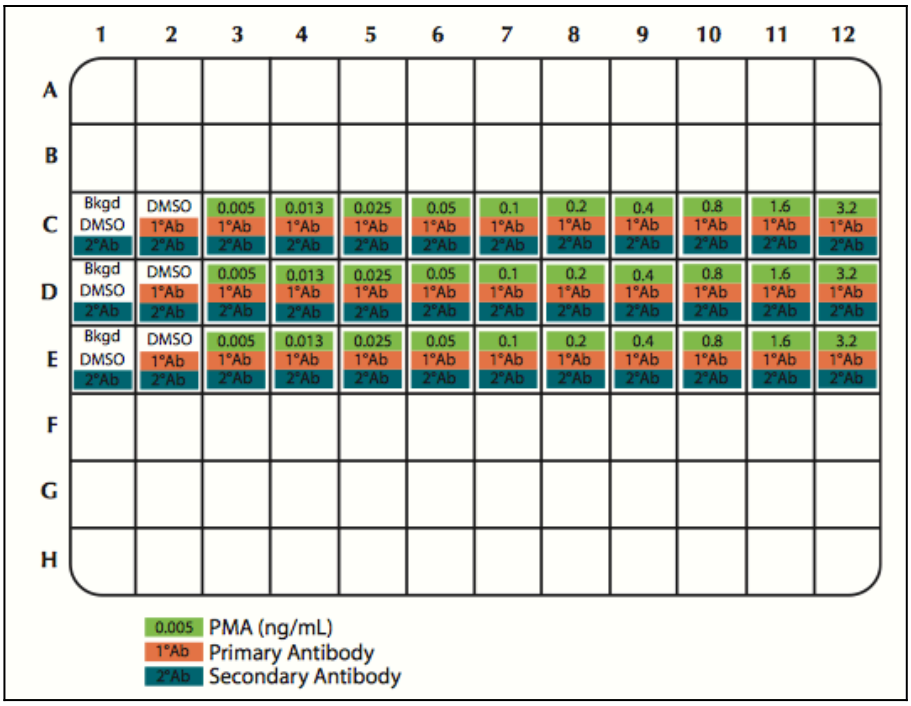

Figure 1. Plate layout of experiment. Round bottom 96-well plate: ~200,000 Jurkat cells per well (C1-E12) in triplicate samples. (Data shown in Figure 2)

Experimental Considerations

Proper selection of microplates can significantly affect results, as each plate has its own characteristics, including well depth, plate autofluorescence, and well-to-well signal crossover. Use the general considerations for microplate selection provided below.

- In-Cell Western analyses use detection at the well surface with minimal liquid present. This results in minimal well-to-well signal spread, allowing the use of both clear as well as black-sided plates with clear bottoms. **Do not use plates with white walls, since autofluorescence from the white surface will create significant noise.**
- Both the flat and round-bottom plates show some plate autofluorescence; however, it is relatively small compared to the actual signal. When using the recommended BD Bioscience round-bottom plate, the recommended focus offset is 3.0 to 3.5 mm. The recommended focus offset for the Nunc round-bottom plate is 3.5 to 3.95 mm.
- **Focus Offset Optimization** - If plates other than those recommended are used, the focus off-set can be determined empirically by scanning a plate containing experimental and control samples using the following focus offset settings.

Instrument	Focus Offset Determination (mm)
Odyssey Classic & Odyssey CLx	1.0, 1.5, 2.0, 2.5, 3.0, 3.5, & 4.0
Odyssey Sa & Aerius	1.5, 2.0, 2.5, 3.0, & 3.95

Use the same intensity settings for each scan. After reviewing the scans, use the focus offset with the highest signal-to-noise ratio for experiments. The actual minimum and maximum focus offset will vary with each instrument. Alternatively, consult the plate manufacturer for the recommended measured distance from the skirt bottom to the bottom of the plate.

- All Aerius and Odyssey® Imaging Systems (excluding Odyssey Fc) require microplates that have a maximum 4.0 mm distance from the base of the microplate to the target detection area of the plate (actual maximum focus offset varies with each Aerius and Odyssey Sa instrument, and is found by choosing Settings > System Administration, then clicking Scanner Information). When using plates specified for In-Cell Western assays, the recommended focus offset is 3.5 mm or higher.
- **Intensity Setting Optimization -**

Instrument	Initial Intensity Setting (700/800 nm)	Intensity Settings: Weak Signal (700/800 nm)	Intensity Settings: Saturated Signal (700/800 nm)
Odyssey® Classic	5 / 5	7.5 / 7.5	2.5 / 2.5
Odyssey CLx	5 / 5	7.5 / 7.5	2.5 / 2.5
	AutoScan*	-	-
Odyssey Sa	7 / 7	8 / 8	4 / 4
Aerius	7 / 7	8 / 8	4 / 4

*The Image Studio™ AutoScan function for the Odyssey CLx alleviates the need to scan the plate at multiple intensity settings.

- Protect plates from light before imaging to ensure highest sensitivity. When storing plates after imaging, the plates should remain protected from light at 4 °C.
- Establish the specificity of your primary antibody by screening lysates through Western blotting. If significant non-specific binding is present, choose alternative primary antibodies. Non-specific binding of primaries will complicate interpretation of In-Cell Western assay results.

Experimental Results

phorbol 12-myristate 13-acetate (ng/mL)

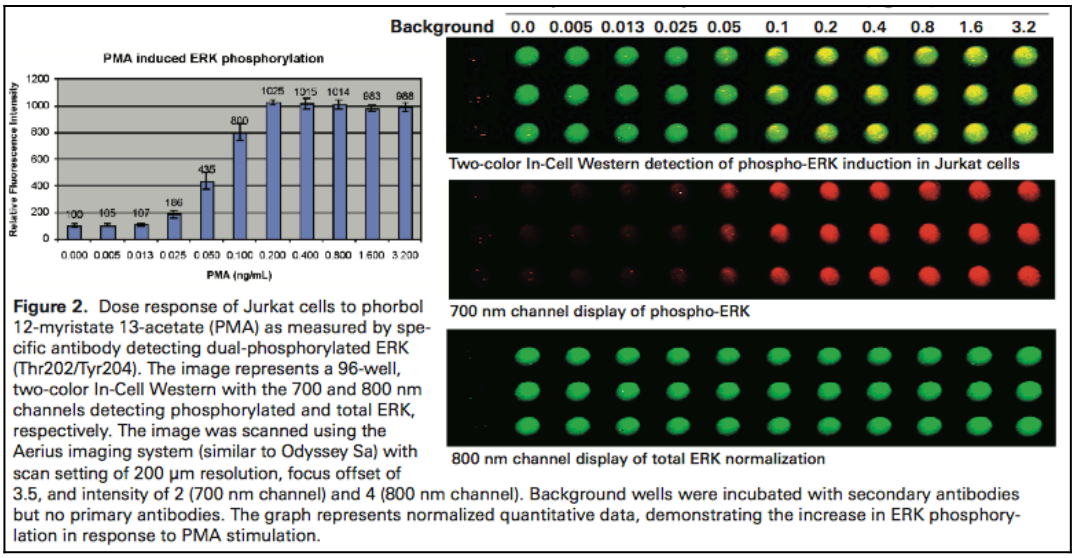

Figure 2. Dose response of Jurkat cells to phorbol 12-myristate 13-acetate (PMA) as measured by specific antibody detecting dual-phosphorylated ERK (Thr202/Tyr204). The image represents a 96-well, two-color In-Cell Western with the 700 and 800 nm channels detecting phosphorylated and total ERK, respectively. The image was scanned using the Aerius imaging system (similar to Odyssey Sa) with scan setting of 200 µm resolution, focus offset of 3.5, and intensity of 2 (700 nm channel) and 4 (800 nm channel). Background wells were incubated with secondary antibodies but no primary antibodies. The graph represents normalized quantitative data, demonstrating the increase in ERK phosphorylation in response to PMA stimulation.

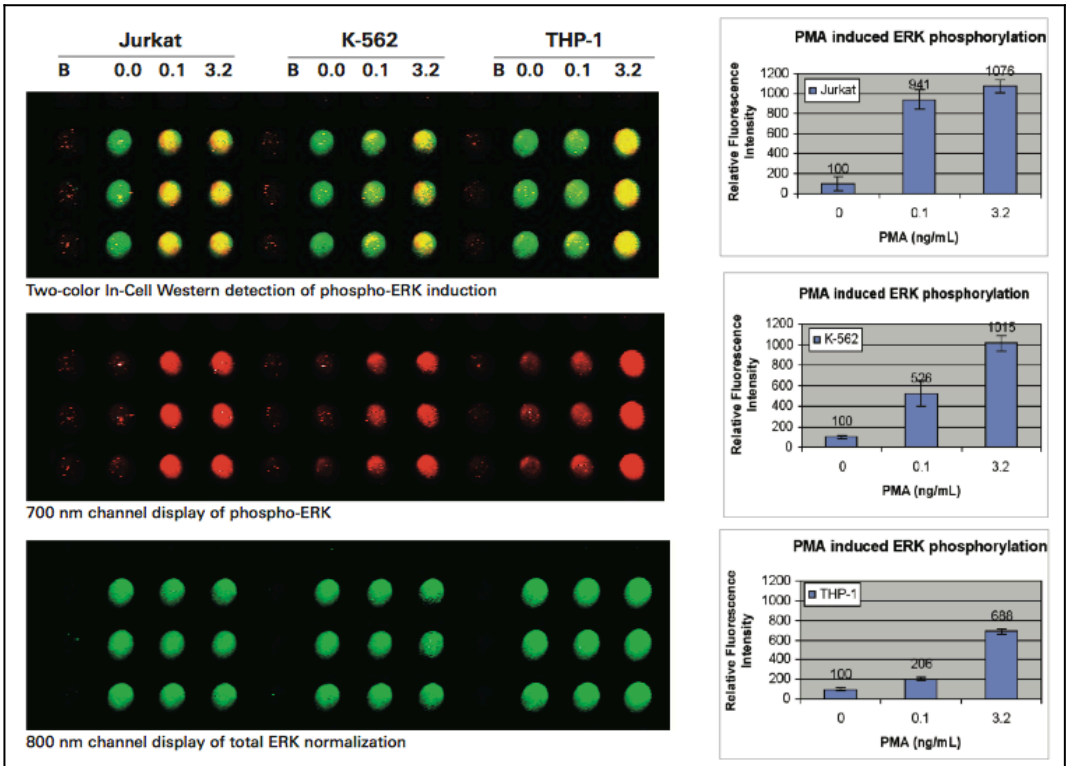

Figure 3. ERK activation in Jurkat, K-562, and THP-1 non-adherent cells in response to PMA as measured by specific antibody detecting dual-phosphorylated ERK (Thr202/Tyr204). The image represents a 96-well, two-color In-Cell Western assay with the 700 and 800 nm channels detecting phosphorylated and total ERK, respectively. The image was scanned using the Aerius Imaging System (similar to Odyssey® Sa) with scan setting of 200 µm resolution, focus offset of 3.5, and intensity of 3.5 (700 nm channel) and 5 (800 nm channel). Background (B) wells were incubated with secondary antibodies but no primary antibodies. The graph represents normalized quantitative data demonstrating the increase in ERK phosphorylation in response to PMA stimulation.

Materials

Jurkat cells, Clone E6-1 TIB-152 by ATCC

Odyssey® Blocking Buffer (PBS) 927-40000 927-40100 by LI-COR

Odyssey Blocking Buffer (TBS) 927-50000 927-50100 by LI-COR

Western Blot Incubation Box 929-97201 929-97205 929-97210 by LI-COR

IRDye® 800CW Goat anti-Mouse Secondary Antibody 925-32210 926-32210 by LI-COR

IRDye 680RD Goat anti-Rabbit Secondary Antibody 925-668071 926-68071 by LI-COR

Normalization antibody: Anti-total ERK1 SC-94 by Santa Cruz Biotechnology

Large Western Incubation Box 929-97301 by LI-COR

THP-1 monocytes TIB-202 by ATCC

K-562 lymphocytes CCL-243 by ATCC

Concentrated Prefer (5X) 411 by Anatech LTP

TO-PRO®-3 T-3605 by Thermo Fisher Scientific

PMA (phorbol 12-myristate 13-acetate) P1585 by Sigma Aldrich

DMSO (dimethyl sulfoxide) D8418 by Sigma Aldrich

pERK mouse antibody 9106 by Cell Signaling

Technology

Protocol

Cell Preparation

Step 1.

Allow Jurkat (ATCC, P/N TIB-152) cell growth in a T75 flask using standard tissue culture procedures. Avoid growing cells to density greater than 2 x 10^6 cells.

NOTES

Lenny Teytelman 20 Jun 2018

Please refer to Figure 1 in the Guidelines for plate layout of experiment.

Cell Preparation

Step 2.

Transfer cells in growth media to 50 mL conical tubes and centrifuge at 500 x g for 5 minutes.

DURATION

00:05:00

Cell Preparation

Step 3.

Remove media and resuspend cell pellet in 10 mL of serum-free media (pre-warmed to 37 °C). **Pipet very slowly in order to maintain cell integrity while disrupting the cell pellet.** Transfer resuspended cells into T75 flask and place in an incubator (37 °C and 5% CO_2).

AMOUNT

10 ml Additional info: pre-warmed serum free media

TEMPERATURE

37 °C Additional info: Incubation

NOTES

James Thornton Jr 15 Dec 2016

IMPORTANT: It is the serum withdrawal from the complete media that allows suspension cells to attach to plates (i.e., T75 flask). Gravity will cause cells to form a monolayer over time (10 to 15 minutes). Once a monolayer is formed, the rest of the cells in the serum-free media will remain in suspension and will not attach further to the plates once a monolayer of cells are established. Only the cells in suspension in the T75 flask will be used in the following steps.

Cell Preparation

Step 4.

Allow cells to settle for 30 minutes before taking a 50µL aliquot of cells for counting using a

hemocytometer.

AMOUNT

50 µl Additional info:
aliquot for counting

DURATION

00:03:00

Cell Preparation

Step 5.

Add appropriate volume of serum-free media so that 1 x 10^6 cells/mL is achieved (1 plate x 96 wells x 200 µL of cells/well = 20 mL/plate).

Cell Preparation

Step 6.

Serum-deprived cells by replacing cells suspended in serum-free media back into the incubator for an additional 3.5 hours or overnight.

DURATION

03:30:00

Cell Treatment

Step 7.

- Add 2 µL of DMSO for both the background samples (serves as non-specific background fluorescence) and resting cells (serves as basal control) in triplicate wells.
- Add 2 µL of 1:1 serial dilutions of PMA ranging from 0.005 to 3.2 ng/mL in triplicate wells.

AMOUNT

2 µl Additional info: DMSO

AMOUNT

2 µl Additional info: of
serial diluted PMA

NOTES

James Thornton Jr 15 Dec 2016

IMPORTANT NOTE about choosing round bottom plates for suspension cell assays: If imaging with the Odyssey® Sa or Aerius Imager, LI-COR recommends clear, round-bottom, 96-well plates from BD Bioscience (P/N 353077) or from Nunc® (P/N 167008). If imaging with the Odyssey CLx or Odyssey Classic Infrared Imager, use plates from BD Bioscience (P/N 353077). Imaging with Nunc round-bottom plates with the Odyssey CLx or Classic Infrared Imager will yield quantitatively accurate results, but the images will not be as visually satisfying and overall signal intensity will be lowered. This is due to differences in the optical properties of the round-bottom wells.

Cell Treatment

Step 8.

Using a multi-channel pipettor, transfer 200 µL of suspended cells (200,000 cells) per well into the wells containing 2 µL of DMSO or PMA from step 6.

AMOUNT

200 µl Additional info:
suspended cells

Cell Treatment

Step 9.

Allow incubation at 37 °C for 15 minutes.

TEMPERATURE

37 °C Additional info:
incubation

DURATION

00:15:00

NOTES

Ashley Humphrey 04 Jun 2018

IMPORTANT: Be careful not to disrupt cells during this PMA-induced activation step. During this critical step, cells will sediment to the bottom of the wells by gravity, forming a monolayer. This monolayer can be easily viewed under a light source. The monolayer will appear opaque rather than transparent. Clumping of cells will lead to detachment from plates during incubation and washing steps. Be careful in handling the plate at this stage because the cells will be very loosely attached to the bottom of the wells.

Fixing/Permeabilizing Cells

Step 10.

Directly add to the cell suspension, 50 µL of concentrated (5X) Prefer (or 25 µL of 37% formaldehyde; final concentration is 4%) into each well.

AMOUNT

50 µl Additional info:
concentrated 5x Prefer

NOTES

James Thornton Jr 15 Dec 2016

IMPORTANT: Gently add Prefer into wells using side of the wells to avoid detaching the cells from the well bottom. During fixation, the cell monolayer will attach more firmly to the wells; however, the strength of the attachment is never as strong as that of adherent cells grown on plates. Thus, a degree of caution is needed during every step of this procedure.

Fixing/Permeabilizing Cells

Step 11.

Allow cells to fix for 20 minutes at room temperature with very gentle rotation (set at speed 2 on The Belly Dancer® (Stovall)).

DURATION

00:20:00

Fixing/Permeabilizing Cells

Step 12.

Centrifuge at 1,500 rpm (332 rcf) for 10 minutes.

DURATION

00:10:00

Fixing/Permeabilizing Cells

Step 13.

To permeabilize cells, wash three times with 100 µL of 1X PBS containing 0.1% Triton® X-100 for 5 minutes each by centrifugation at 1,500 rpm (332 rcf). Detailed in steps 14-24.

AMOUNT

100 µl Additional info: 1x
PBS

NOTES

James Thornton Jr 11 Jan 2017

Do not allow cells/wells to become dry during washing. Immediately add the next wash after manual disposal.

James Thornton Jr 11 Jan 2017

NOTE: If detecting cell surface proteins with exofacial antigens, you do not need to permeabilize cells.

Fixing/Permeabilizing Cells

Step 14.

Prepare Triton Permeabilization Solution as follows:

1X PBS	495 mL
10% Triton X-100	5 mL
1X PBS + 0.1% Triton X-100	500 mL

Fixing/Permeabilizing Cells

Step 15.

Remove fixing solution (if using formaldehyde, collect in an appropriate waste container).

Fixing/Permeabilizing Cells

Step 16.

Using a multi-channel pipettor, add 100 µL of fresh *Triton Permeabilization Solution*. **Add the solution by pipetting down the sides of the wells carefully to avoid detaching the cells from the well bottom.** (wash 1/3)

AMOUNT

100 µl Additional info:
Triton Permeabilization

Solution

Fixing/Permeabilizing Cells

Step 17.

Centrifuge at 1,500 rpm (332 rcf) for 5 minutes. (wash 1/3)

DURATION

00:05:00

Fixing/Permeabilizing Cells

Step 18.

Gently remove Triton Permeabilization Solution by manually pipetting. (wash 1/3)

Fixing/Permeabilizing Cells

Step 19.

Using a multi-channel pipettor, add 100 µL of fresh *Triton Permeabilization Solution.* **Add the solution by pipetting down the sides of the wells carefully to avoid detaching the cells from the well bottom.** (wash 2/3)

AMOUNT

100 µl Additional info:
Triton Permeabilization
Solution

Fixing/Permeabilizing Cells

Step 20.

Centrifuge at 1,500 rpm (332 rcf) for 5 minutes. (wash 2/3)

DURATION

00:05:00

Fixing/Permeabilizing Cells

Step 21.

Gently remove Triton Permeabilization Solution by manually pipetting. (wash 2/3)

Fixing/Permeabilizing Cells

Step 22.

Using a multi-channel pipettor, add 100 µL of fresh *Triton Permeabilization Solution*. **Add the solution by pipetting down the sides of the wells carefully to avoid detaching the cells from the well bottom.** (wash 3/3)

AMOUNT

100 µl Additional info:
Triton Permeabilization
Solution

Fixing/Permeabilizing Cells

Step 23.

Centrifuge at 1,500 rpm (332 rcf) for 5 minutes. (wash 3/3)

DURATION

00:05:00

Fixing/Permeabilizing Cells

Step 24.

Gently remove Triton Permeabilization Solution by manually pipetting. (wash 3/3)

Blocking Cells

Step 25.

Using a multi-channel pipettor, block cells/wells by adding 100 µL of Odyssey Blocking Buffer to each well. **Add the solution by pipetting down the sides of the wells carefully to avoid detaching the cells.**

AMOUNT

100 µl Additional info:
Odyssey Blocking Buffer

Blocking Cells

Step 26.

Allow blocking for 1 hour at room temperature with very gentle shaking on a plate shaker.

DURATION

01:00:00

Primary Antibodies

Step 27.

Dilute the two primary antibodies in Odyssey Blocking Buffer. Combine the following antibodies for ERK target analysis:

- Rabbit anti-ERK antibody (1:200 dilution; Santa Cruz)
- Mouse anti-phospho-ERK antibody (1:100 dilution; Cell Signaling Technology)

Primary Antibodies

Step 28.

Mix the primary antibody solution well before adding to wells.

Primary Antibodies

Step 29.

Remove blocking buffer from the blocking step.

Primary Antibodies

Step 30.

Add 50 µL of Odyssey Blocking Buffer to the background control wells only (serves as non-specific background fluorescence).

AMOUNT

50 µl Additional info:
Odyssey Blocking Buffer

Primary Antibodies

Step 31.

Add 50 µL of the primary antibody solution into rest of wells.

AMOUNT

50 µl Additional info:
primary antibody solution

Primary Antibodies

Step 32.

Incubate with primary antibody for 2 hours at room temperature or overnight at 4 °C with very gentle shaking on a plate shaker.

DURATION

02:00:00

Primary Antibodies

Step 33.

Wash the plates five times with 200 µL of 1X PBS + 0.1% Tween® 20 for 5 minutes by centrifugation at 1,500 rpm (332 rcf). Detailed in steps 34-50.

Primary Antibodies

Step 34.

Prepare Tween® Washing Solution as follows:

1X PBS	995 mL
20% Tween 20	5 mL
1X PBS with 0.1% Tween 20	1,000 mL

Primary Antibodies

Step 35.

Remove primary antibody solution.

Primary Antibodies

Step 36.

Using a multi-channel pipettor, add 200 µL of *Tween Washing Solution*. **Make sure to add the solution down the sides of the wells carefully to avoid detaching the cells**. (wash 1/5)

AMOUNT

200 µl Additional info:
Tween Washing Solution

Primary Antibodies

Step 37.

Centrifuge at 1,500 rpm (332 rcf) for 5 minutes. (wash 1/5)

DURATION

00:05:00

Primary Antibodies

Step 38.

Gently remove Tween Washing Solution by manually pipetting. (wash 1/5)

Primary Antibodies

Step 39.

Using a multi-channel pipettor, add 200 µL of *Tween Washing Solution*. **Make sure to add the solution down the sides of the wells carefully to avoid detaching the cells**. (wash 2/5)

AMOUNT

200 µl Additional info:
Tween Washing Solution

Primary Antibodies

Step 40.

Centrifuge at 1,500 rpm (332 rcf) for 5 minutes. (wash 2/5)

DURATION

00:05:00

Primary Antibodies

Step 41.

Gently remove Tween Washing Solution by manually pipetting. (wash 2/5)

Primary Antibodies

Step 42.

Using a multi-channel pipettor, add 200 µL of *Tween Washing Solution.* **Make sure to add the solution down the sides of the wells carefully to avoid detaching the cells**. (wash 3/5)

AMOUNT

200 µl Additional info:
Tween Washing Solution

Primary Antibodies

Step 43.

Centrifuge at 1,500 rpm (332 rcf) for 5 minutes. (wash 3/5)

DURATION

00:05:00

Primary Antibodies

Step 44.

Gently remove Tween Washing Solution by manually pipetting. (wash 3/5)

Primary Antibodies

Step 45.

Using a multi-channel pipettor, add 200 µL of *Tween Washing Solution*. **Make sure to add the solution down the sides of the wells carefully to avoid detaching the cells**. (wash 4/5)

AMOUNT

200 µl Additional info:
Tween Washing Solution

Primary Antibodies

Step 46.

Centrifuge at 1,500 rpm (332 rcf) for 5 minutes. (wash 4/5)

DURATION

00:05:00

Primary Antibodies

Step 47.

Gently remove Tween Washing Solution by manually pipetting. (wash 4/5)

Primary Antibodies

Step 48.

Using a multi-channel pipettor, add 200 µL of *Tween Washing Solution*. **Make sure to add the solution down the sides of the wells carefully to avoid detaching the cells**. (wash 5/5)

AMOUNT

200 µl Additional info:
Tween Washing Solution

Primary Antibodies

Step 49.

Centrifuge at 1,500 rpm (332 rcf) for 5 minutes. (wash 5/5)

DURATION

00:05:00

Primary Antibodies

Step 50.

Gently remove Tween Washing Solution by manually pipetting. (wash 5/5)

Secondary Antibodies

Step 51.

Dilute the fluorescently-labeled secondary antibody in Odyssey® Blocking Buffer with 0.2% Tween 20 in order to lower background as specified below.

- IRDye® 680RD Goat anti-Mouse (1:800 dilution)
- IRDye 800CW Goat anti-Rabbit (1:800 dilution)

NOTES

James Thornton Jr 11 Jan 2017

Recommended dilution range is 1:200 to 1:1,200.

James Thornton Jr 11 Jan 2017

Avoid prolonged exposure of the antibody vials to light.

Secondary Antibodies

Step 52.

- Mix the antibody solutions thoroughly and add 50 µL of the secondary antibody solution to each well.
- Incubate for one hour with very gentle shaking on a rotator at room temperature.
- Protect plate from light during incubation. Use a large black Western Incubation Box to protect plate from light during subsequent steps.

AMOUNT

50 µl Additional info:
Secondary antibody
solution

Secondary Antibodies

Step 53.

Wash the plates 5 times with 200 µL of 1X PBS + 0.1% Tween 20 at room temperature for 5 minutes by centrifugation at 1,500 rpm (332 rcf). Detailed in steps 54-69.

NOTES

James Thornton Jr 11 Jan 2017

Protect plate from light during washing.

Secondary Antibodies

Step 54.

Remove secondary antibody solution.

Secondary Antibodies

Step 55.

Using a multi-channel pipettor, add 200 µL of *Tween Washing Solution*. **Make sure to add the solution down the sides of the wells carefully to avoid detaching the cells**. (wash 1/5)

AMOUNT

200 µl Additional info:
Tween Washing Solution

Secondary Antibodies

Step 56.

Centrifuge at 1,500 rpm (332 rcf) for 5 minutes. (wash 1/5)

DURATION

00:05:00

Secondary Antibodies

Step 57.

Gently remove Tween Washing Solution by manually pipetting. (wash 1/5)

Secondary Antibodies

Step 58.

Using a multi-channel pipettor, add 200 µL of *Tween Washing Solution*. **Make sure to add the solution down the sides of the wells carefully to avoid detaching the cells**. (wash 2/5)

AMOUNT

200 µl Additional info:
Tween Washing Solution

Secondary Antibodies

Step 59.

Centrifuge at 1,500 rpm (332 rcf) for 5 minutes. (wash 2/5)

DURATION

00:05:00

Secondary Antibodies

Step 60.

Gently remove Tween Washing Solution by manually pipetting. (wash 2/5)

Secondary Antibodies

Step 61.

Using a multi-channel pipettor, add 200 µL of *Tween Washing Solution*. **Make sure to add the solution down the sides of the wells carefully to avoid detaching the cells**. (wash 3/5)

AMOUNT

200 µl Additional info:
Tween Washing Solution

Secondary Antibodies

Step 62.

Centrifuge at 1,500 rpm (332 rcf) for 5 minutes. (wash 3/5)

DURATION

00:05:00

Secondary Antibodies

Step 63.

Gently remove Tween Washing Solution by manually pipetting. (wash 3/5)

Secondary Antibodies

Step 64.

Using a multi-channel pipettor, add 200 µL of *Tween Washing Solution*. **Make sure to add the solution down the sides of the wells carefully to avoid detaching the cells**. (wash 4/5)

AMOUNT

200 µl Additional info:
Tween Washing Solution

Secondary Antibodies

Step 65.

Centrifuge at 1,500 rpm (332 rcf) for 5 minutes. (wash 4/5)

DURATION

00:05:00

Secondary Antibodies

Step 66.

Gently remove Tween Washing Solution by manually pipetting. (wash 4/5)

Secondary Antibodies

Step 67.

Using a multi-channel pipettor, add 200 µL of *Tween Washing Solution*. **Make sure to add the solution down the sides of the wells carefully to avoid detaching the cells**. (wash 5/5)

AMOUNT

200 µl Additional info:
Tween Washing Solution

Secondary Antibodies

Step 68.
Centrifuge at 1,500 rpm (332 rcf) for 5 minutes. (wash 5/5)

Secondary Antibodies

Step 69.
Gently remove Tween Washing Solution by manually pipetting. (wash 5/5)

Imaging

Step 70.
After final wash, remove wash solution completely from wells. For best results, scan plate immediately; plates may also be stored at 4 °C for several weeks (protected from light).

TEMPERATURE

4 °C Additional info:
storage temperature

Imaging

Step 71.
Scan plate with detection in both 700 and 800 nm channels using an Odyssey or Aerius System described following:
69 µm

Instrument	Resolution*	Focus Offset	Scan Quality*	Intensity Setting (700/800)	Scan Time Medium Quality
Odyssey Classic	169 µm	3.5	medium-lowest	5 / 5	7 min
Odyssey CLx	169 µm	3.5	medium-lowest	5 / 5	7 min
	169 µm	3.5	medium-lowest	AutoScan	16 min
Odyssey Sa	200 µm	3.5	medium-lowest	7 / 7	3 min
Aerius	200 µm	3.5	medium-lowest	7 / 7	3 min

**Higher resolution or scan quality may be used, but scan time will increase.*

NOTES

Margaret Dentlinger 20 Jun 2018
All settings may require adjustment for optimal data quality (Imaging Sections)

Warnings

See SDS for safety and warnings.