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QuantiGene multiplex assay

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ABSTRACT

This protocol outlines a method of simultaneously measuring the expression of up to 80 genes in any one sample using the QuantiGene multiplex assay from ThermoFisher. This protocol was adapted from the lab of Professor Gill Bates. Cells are grown in 96-well plates, lysed, snap frozen and stored at -80^{o}C. Lysed samples are then incubated overnight with magnetic capture beads as well as a probe panel to detect a custom designed set of genes. A series of incubations and washes amplify the captured RNA signals, which are subsequently measured using a fluorescent probe on a Magpix (Luminex).

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MATERIALS TEXT

Normal cell culture materials

- Media

QuantiGene reagents

- Lysis mixture
- Proteinase K
- Blocking reagent
- Pre-amplifier solution
- Amplifier solution
- Label probe solution
- SAPE
- SAPE diluent
- Wash buffer component 1
- Wash buffer component 2
- SAPE wash buffer
- Magnetic separation plate
- Plate seals
- Hybridization plate
- Pressure seals
- Probe set
- Capture beads

Lab equipment

- 200ul multichannel pipette
- Reagent reservoir
- Vortemp shaking incubator
- Magpix plate reader
- Handheld magnetic plate washer
- RNase-free water
- Dry ice

Sample preparation

1 Pre-warm lysis mixture at **37°C** for **30 mins**, followed by gentle swirling. **00:30:00** 30m

2 Prepare **working lysis mixture** by adding **10 µL** of **proteinase K** to each **1 mL** of **lysis mixture** required.

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3 Add **1/2 volume** of working lysis mixture to cells in **culture media** from a reagent reservoir using the multichannel pipette (e.g. for a 96 well plate containing **100 µL** of media per well, add **50 µL** of working lysis mixture).

4 **Mix** by pipetting up and down 3-4 times, discard the tips before continuing with the consecutive wells (more is more).

5 **Snap freeze** on a bed of **dry ice** and store in the **-80°C** until required.

6 When required, incubate the cell culture plate in the **Vortemp** pre-warmed to **50°C** for **01:00:00** without shaking. 1h

7 **Verify** cell lysis using the cell culture microscope.

7.1 For new QuantiGene plexes, appropriate dilutions must be ascertained via a serial dilution experiment- refer to Papadopoulou et al, 2019 and pages **29–30** of the **QuantiGene Plex Gene Expression Assay User Guide** for more details.

Storage of lysates

8 Samples should be stored long term in the **-80°C** freezer. Samples do not need to be thawed on ice and are **stable** at **RT.**

Assay day 1

9 **0 µL** Pre-warm **lysis mixture** at **37°C** for **00:30:00** followed by gentle swirling. 30m

10 Arrange sufficient **pre-vortexed** and **diluted** samples (for **experiment**) or **serial dilutions** and **reference RNA** (for plex **optimisation**) as per your plate plan and keep at **RT-** each **40 µL** sample should be run in **duplicate,** include a **background control** by making sufficient diluted lysis mixture (1 volume lysis mixture plus 2 volumes of RNase-free water).

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11 Handle the reagents listed below as follows:

11.1 **Probe Set & Blocking Reagent** (kept at **-20°C** in QuantiGene reagents box): **thaw** and **vortex** briefly to mix, then **centrifuge probe set** briefly to collect contents at the bottom of the tube.

11.2 **Proteinase K** (kept at **-20°C** in QuantiGene reagents box): keep on **ice**.

11.3 **Capture Beads** (kept at **4°C** in QuantiGene capture bead box): take out of storage right before use and **protect** from **light**.

12 Prepare an appropriate volume of **working bead mix** by combining the following reagents in the order listed (**this is for 2- to 64-plex assays**), scale according to the number of wells on your QuantiGene plate(s), keep working bead mix at RT and protected from light.

12.1

A	B	C	D
Order	**Reagent**	**1 well (µl)**	**96 wells (+14 for extra) (µl)**
1	Nuclease-free water	2.6	286
2	Lysis Mixture	3.3	363
3	Blocking Reagent	1	110
4	Proteinase K	0.1	11
5	Capture Beads (vortex for 30 seconds before adding)	0.5	55
6	Probe Set	2.5	275
TOTAL:	**10**	**1100**	

Working bead mix

13 Vortex **Working Bead Mix** for **10 seconds** and then carefully pipette **10µl** into each well of a **magnetic separation plate**, avoiding bubbles, add **40 µL** of each sample (including background controls) as per your plate plan into the magnetic separation plate (load each sample with a new pipette tip).

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14 **Seal** magnetic separation plate with a pressure seal. Use the backing of the pressure seal to firmly and evenly apply pressure across the whole seal and lastly run your finger along each edge of the plate to seal.

15 Place the magnetic separation plate in the Vortemp shaking incubator for **18:00:00** to **22:00:00** at **54°C** at **600rpm.** 1d 16h

Assay day 2 5h 17m 15s

16 Turn on **Magpix** and computer to allow lasers time to **warm up.**

17 Warm **pre-amplifier solution**, **amplifier solution**, **label probe solution** and **SAPE diluent** at **37°C** at least **00:30:00** prior to use. 30m

18 Prepare **1 x wash buffer** by adding **3 mL** **wash buffer component 1** and **50 mL** **wash buffer component 2** and topping up to **1 L** with **nuclease-free water** from the Milli-Q or Hyclone water.

19 **Remove** the magnetic **separation plate** from the shaking incubator and adjust temperature to **51°C** at **600rpm**.

20 **Centrifuge** magnetic separation plate at **240 × g** for **00:01:00** at RT. 1m

21 In the **fume hood,** insert magnetic separation plate into **handheld magnetic plate washer** and ensure it is securely locked, allow **00:01:00** to allow magnetic beads to accumulate on bottom of each well. 1m

22 Keep plate inserted in handheld magnetic plate washer at all times for this step: add **100 µL** of **1 x wash buffer**, wait **00:00:15** to allow the magnetic beads to accumulate at the bottom of each well, remove solution by quickly **inverting** over a waste container and **gently blot** on several layers of paper towel to remove residual solution. 15s

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23 Repeat previous step **two more times.**

24 Add **50 µL** of **pre-amplifier solution** to each well and **seal** with a **foil plate seal**, return to Vortemp and incubate for **01:00:00** at **51°C** at **600rpm** (the minimum time for incubation is **00:45:00** and maximum is **02:00:00**). 3h 45m

25 **Repeat** steps **21-24** for **amplifier** solution in place of **pre-amplifier** solution.

26 **Repeat** steps **21-24** for **label probe** solution in place of **pre-amplifier** solution.

27 Prepare **SAPE working reagent** by mixing **3 µL** of **SAPE** to **1 mL** **SAPE diluent** (scaled to the number of the **wells** on your plates + **10%**), **vortex** to mix and keep at **RT protected** from **light.**

28 **Repeat** steps **21-24** then add **50 µL** of **SAPE working reagent** to each well and **seal** with a foil plate deal, return to Vortemp and incubate for **00:30:00** at **51°C** at **600 rpm** before turning off Vortemp. **Do not exceed** **00:30:00** **incubation.** 1h

29 **Repeat** steps **21-24** with **SAPE wash buffer** in place of regular wash buffer**.**

30 To prepare the plate for analysis, add **130 µL** of **SAPE wash buffer** to each well, **seal** the plate with a foil plate seal, **tape** sealed magnetic separation plate down onto a **shaker** and shake **vigorously** (~**800rpm**) at **RT,** immediately run plate on **Magpix.**

31 If analysing more than one plate, keep consecutive plates at room temperature protected from light until required and then prepare as in step 32 and shake vigorously (~800rpm) at RT before analysing on the Magpix instrument.

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32 Plates can be stored **long-term** in at **4°C,** for reanalysis of stored plates, repeat steps 29-30.

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