Technical Note

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Guidelines and Procedure Modifications for Running QuantiGene ViewRNA ISH Cell Assay in a 96-Well Plate Format

About this Technical Note

Guidelines, procedures and procedure modification are provided for running the QuantiGene ViewRNA ISH Cell Assay in a 96-well assay format. This technical note should be used in conjunction with the *QuantiGene ViewRNA ISH Cell Assay User Manual*.

One QuantiGene ViewRNA ISH Cell Assay Kit is sufficient for running 144 wells (1.5 plates) in the 96-well format.

Required Materials

Table 1 Required Materials for Running a QuantiGene ViewRNA ISH Assay in a 96-Well Format

Item	Source
96-well optical bottom plate	Major Laboratory Supplier (MLS)
Optional. Vacuum aspirator and 8-channel adapter	Corning P/N 4930 and 4931
1X PBS, sterile cell culture grade (required for sample preparation procedures)	MLS

Important Procedural Notes and Guidelines

- Adherent cells must be grown and fixed on a ploy-L-lysine coated 96-well plate.
- This assay is compatible with both glass-bottom and polystyrene plates that are qualified for imaging studies. For best image quality (highest signal-to-noise ratios), the glass-bottomed plate is strongly recommended.
- This assay should be run with a minimum of 24 wells/experiment, in the 96-well format, to avoid reagent shortage.
- Aspiration and dispensing of contents from and into the wells should be performed slowly and gently to reduce cell loss. Well contents should be removed slowly against the well wall and added against the upper well wall. For optimal cell retention, well contents can be removed using a 200 µL multichannel pipette or an aspirator with an 8-channel adaptor connected to a vacuum.

Sample Preparation Procedures

Preparing Poly-L-Lysine Coated Plates

To prepare a poly-L-lysine coated 96-well plate:

1. Dilute poly-L-lysine 1:10 (final concentration of 0.01%) by adding 1.1 mL of poly-L-lysine stock solution to 9.9 mL of room temperature (RT) nuclease-free water. Mix well.
2. Dispense 100 µL/well of diluted poly-L-lysine and incubate for 15 minutes at RT.
3. Expel the poly-L-lysine by inverting the plate over an appropriate receptacle and assertively flicking the solution from the wells. Wash three times, each with 150 µL/well of 1X PBS.
4. Expel the final 1X PBS wash. Allow to air dry, with lid off, in a laminar flow tissue culture hood overnight. Keep air flow on and turn UV light off.
5. Coated plate may be used immediately for cell seeding or stored at 4 °C under sterile conditions. Coated plates can be stored up to 1 month.
6. Proceed to culturing cells on poly-L-Lysine coated plate.

Culturing Adherent Cells

To culture fresh adherent cells in a poly-L-lysine coated 96-well plate:

1. Wash the adherent cells with 1X PBS and trypsinize. Resuspend the cells in complete cell culture medium.

2. Pellet the cells (200 x g, 5 minutes) at RT, remove the supernatant, and resuspend the cell pellet in fresh complete culture medium.
3. Count and adjust the cell density such that when seeded, 100 µL/well, the culture will reach 70-90% confluency at the start of the assay.
4. Dispense 100 µL/well of the adjusted cell suspension.
5. Incubate the plate overnight under the recommended growth conditions for the cells.
6. Proceed to fixation of cells.

Fixing the Cells

To fix cells in the 96-well plate:

1. *For one 96 well plate.* In a fume hood, prepare 6.5 mL of fresh 4% formaldehyde solution by diluting 700 µL of a 37% stock formaldehyde with 5.8 mL of 1X PBS and mixing well.
2. Using a multichannel pipette, gently remove the culture medium from the wells and wash cells twice, each with 150 µL /well of 1X PBS.
3. Remove the final 1X PBS wash and dispense 60 µL/well of freshly prepared 4% formaldehyde solution. Cover plate with lid and incubate at RT for 30 minutes.
4. Using a multichannel pipette, gently remove the formaldehyde solution from the wells and wash cells three times, each with 150 µL/well of 1X PBS.
5. Fixed cells may be used immediately for *in situ* assay, in which case, proceed to the assay setup section of the *QuantiGene ViewRNA ISH Cell Assay User Manual*.
6. If not using immediately, samples can be dehydrated for long term storage.

IMPORTANT: Sample dehydration and rehydration on glass-bottom plates tend to weaken the adhesive between the glass bottom and the polystyrene plate. Hence, plates may become leaky.

A. Remove the final 1X PBS wash and replace with 150 µL/well of 50% ethanol. Incubate for 2 minutes at RT.
B. Remove the 50% ethanol and replace with 150 µL/well of 70% ethanol. Incubate for 2 minutes at RT.
C. Remove the 70% ethanol and replace with 150 µL/well of 100% ethanol. Incubate for 2 minutes at RT.
D. Remove the 100% ethanol and replace with 150 µL/well of fresh 100% ethanol.
E. Seal the plate with parafilm and store the dehydrated cells in 100% ethanol at –20 °C until needed. Dehydrated cells can be stored under these conditions for several weeks.

NOTE: Dehydrated cells must be rehydrated prior to being used in the *in situ* assay.

Assay Procedure Modifications

- If rehydration is required, modify volumes to 150 µL/well.
- All reagent additions should be modified to 60 µL/well.
- All wash step volumes (1X PBS or Wash Buffer) should be modified to 150 µL/well.

Technical Help

For technical support, contact the appropriate resource provided below based on your geographical location. For an updated list of FAQs and product support literature, visit our website at www.affymetrix.com/panomics.

Table 2 Technical Support Contacts

Location	Contact Information
North America	1.877.726.6642 option 1, then option 3; pqbhelp@affymetrix.com
Europe	+44 1628-552550; techsupport_europe@affymetrix.com
Asia	+81 3 6430 430; techsupport_asia@affymetrix.com

US Headquarters
Affymetrix, Inc.
3420 Central Expressway
Santa Clara, CA 95051
Tel: 1-888-362-2447
Direct: 1-408-731-5000
Fax: 1-408-731-5380
Email: info@affymetrix.com
Email: orders_fremont@affymetrix.com
Email: pqbhelp@affymetrix.com

European Headquarters
Panomics Srl
Via Sardegna 1
20060 Vignate-Milano (Italy)
Tel: +39-02-95-360-250
Fax: +39-02-95-360-992
Email: info_europe@affymetrix.com
Email: order_europe@affymetrix.com
Email: techsupport_europe@affymetrix.com

Asia Pacific Headquarters
Affymetrix Singapore Pte Ltd.
No.7 Gul Circle #2M-01/08
Keppel Logistics Building
Singapore 629563
Tel: +65-6395-7200
Fax: +65-6395-7300
Email: info_asia@affymetrix.com
Email: order_asia@affymetrix.com
Email: techsupport_asia@affymetrix.com

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