Quick Reference Guide

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# QuantiGene ViewRNA miRNA ISH Cell Assay

## 24-Well Format for Suspension Cells

**IMPORTANT: If running QuantiGene ViewRNA miRNA ISH Cell Assay for the first time, please refer to the appropriate *QuantiGene ViewRNA miRNA ISH Cell Assay User Manual*, based on your sample type and processing format, to review assay guidelines and detailed procedures.**

## Part 1: Sample Preparation

| Step | Task |
|---|---|
| **Step 1. Coat Glass Coverslips with Poly-L-Lysine**<br>**1 hr** | ▪ Prepare 25 mL of 0.01% poly-L-lysine solution<br>▪ Ensure availability of 100% ethanol, sterile 1X PBS, 24-well tissue culture plate, 12-mm glass coverslips, fine-tipped forceps<br>▪ Sterilize coverslips in 24-well plate with 100% ethanol for 15 min at RT<br>▪ Remove ethanol and air dry coverslips completely for 15 min at RT<br>▪ Coat coverslips with diluted poly-L-lysine for 15 min at RT<br>▪ Wash coverslips 3 times in 1X PBS<br>▪ Air dry coated coverslips O/N at RT in laminar flow hood |
| **Step 2. Culture Suspension Cells**<br>**20 min** | ▪ Culture cells under the recommended growth conditions until confluent |

## Part 2: Pretreatment and Target Probe(s) Hybridization

| Step | Task |
|---|---|
| **Step 3. Fix Samples**<br>**1 hr 15 min** | ▪ Prepare 1 L of 1X PBS, 4.5 mL of 4% formaldehyde in 1X PBS<br>▪ Pellet cells, remove culture medium and wash once with 1X PBS<br>▪ Fix cells in suspension with 4% formaldehyde for 1 hr at RT (in a fume hood), mix every 20 min<br>▪ Pellet and wash cells 3 times with 1X PBS<br>▪ Adjust cell concentration to 1000 cells/µL with fresh 1X PBS<br>▪ Spot and evenly distribute 20,000 cells per poly-L-lysine coated glass coverslip<br>▪ Bake at 50 °C for 30 min or until cells are completely dried |
| **Step 4. *Optional.* Dehydrate/ Rehydrate**<br>**10-20 min** | ▪ Ensure availability of 50%, 70%, and 100% ethanol if dehydrating cells for storage or for rehydrating cells for the assay<br>▪ Dehydrate cells if storing sample: 100% EtOH and store at –20 °C<br>▪ Rehydrate cells for assay if starting with dehydrated sample: 100% EtOH, 2 min -> 70% EtOH, 2 min -> 50% EtOH, 2 min -> 1X PBS, 10 min |
| **Step 5. Prepare Buffers and Reagents during Sample Fixation**<br>**10 min** | ▪ Set a dry incubator to 40 ± 1 °C<br>▪ Equilibrate EDC to RT<br>▪ Pre-warm Probe Set Diluent QF to 40 °C<br>▪ Thaw Probe Set(s) and place on ice<br>▪ Place Protease QS on ice<br>▪ Prepare 800 mL of Wash Buffer, 30 mL Storage Buffer<br>▪ Ensure availability of Cross-Linking Buffer QM, Detergent Solution QC |
| **Step 6. Cross-Link with EDC**<br>**1 hr 30 min** | ▪ Equilibrate cells with Cross-Linking Buffer QM 2 times, 10 min each at RT with rocking<br>▪ Prepare 0.16M EDC just before use<br>▪ Cross-link target with 0.16 M EDC for 1 hr at RT, rocking<br>▪ Wash cells 3 times with 1X PBS |

| Step | Task |
|---|---|
| **Step 7. *Optional.* Dehydrate/ Rehydrate**<br>**10-20 min** | ▪ Dehydrate cells if storing sample: 50% EtOH 2 min -> 70% EtOH, 2 min -> 100% EtOH, 2 min -> Fresh 100% EtOH and store at –20 °C<br>▪ Rehydrate cells for assay if starting with dehydrated sample: 100% EtOH, 2 min -> 70% EtOH, 2 min -> 50% EtOH, 2 min -> 1X PBS, 10 min |
| **Step 8. Permeabilize and Digest with Protease**<br>**40 min** | ▪ Permeabilize cells with Detergent Solution QC for 10 min at RT<br>▪ Wash cells 2 times with 1X PBS<br>▪ Prepare Working Protease Solution<br>▪ Treat cells with Working Protease Solution for 10 min at RT<br>▪ Wash cells 3X with 1X PBS |
| **Step 9. Hybridize Target Probe(s)**<br>**3 hrs** | ▪ Prepare Working Probe Set(s) Solutions<br>▪ Hybridize cells with Working Probe Solutions for 3 hrs at 40 °C |
| **Step 10. Wash Samples**<br>**10 min** | ▪ Wash cells 3 times in Wash Buffer, soaking 3 min each |
| **Step 11. Stop Point**<br>**1 min** | ▪ Store samples in Storage Buffer, covered with lid and seal with parafilm at 4 °C. Do not exceed 24 hrs |

## Part 3. Signal Amplification and Detection

| Step | Task |
|---|---|
| **Step 12. Prepare Additional Buffers and Reagents**<br>**10 min** | ▪ Prewarm PreAmplifier Mix QM, Amplifier Mix QM, and Label Probe Diluent QF to 40 °C<br>▪ Equilibrate the following reagents to RT:<br>▫ AP Enhancer, Fast Red Tablets, Naphthol Buffer<br>▫ Cell samples<br>▪ Thaw, mix and place on ice 100X DAPI and Label Probe 4-Alexa 488<br>▪ Prepare 11 mL 1X DAPI and store on ice protected from light<br>▪ Place Label Probe 1-AP on ice<br>▪ Ensure availability of Wash Buffer and 1X PBS<br>▪ Ensure availability of Prolong® Gold Antifade Reagent |
| **Step 13. Wash Storage Buffer off Samples**<br>**5 min** | ▪ Wash cells 2X with Wash Buffer |
| **Step 14. Hybridize with PreAmplifier**<br>**1 hr** | ▪ Prepare Working PreAmplifier Mix Solution<br>▪ Hybridize cells with Working PreAmplifier Mix Solution for 1 hr at 40 °C |
| **Step 15. Wash Samples**<br>**10 min** | ▪ Wash cells 3X with Wash Buffer, soaking 3 min each |
| **Step 16. Hybridize with Amplifier**<br>**1 hr** | ▪ Prepare Working Amplifier Mix Solution<br>▪ Hybridize cells with Working Amplifier Mix Solution for 1 hr at 40 °C |
| **Step 17. Wash Samples**<br>**10 min** | ▪ Wash cells 3X with Wash Buffer, soaking 3 min each |
| **Step 18. Hybridize with Label Probe(s)**<br>**1 hr** | ▪ Prepare Label Probe(s) Working Solution<br>▪ Hybridize cells with Label Probe(s) Working Solution for 1 hr at 40 °C |
| **Step 19. Wash Samples**<br>**20 min** | ▪ Wash cells 3 times in Wash Buffer, soaking 3 min each for the first 2 washes and 10 min for the final wash |

| Step | Task |
|---|---|
| **Step 20. Fast Red Development**<br>**1 hr 20 min** | ▪ Incubate cells with AP-Enhancer for 5 min at RT; prepare Fast Red Substrate<br>▪ Remove AP-Enhancer, add Fast Red Substrate and incubate at 40 °C for 45 min<br>▪ Wash cells 2 times with 1X PBS<br>▪ Fix cells with 4% formaldehyde for 10 min at RT (in a fume hood)<br>▪ Wash cells 3 times with 1X PBS |
| **Step 21. DAPI Counter Stain**<br>**5 min** | ▪ Stain cells with 1X DAPI for 5 min at RT<br>▪ Wash cells once with 1X PBS and replace with fresh 1X PBS |
| **Step 22. Mount Samples on Glass Slides**<br>**15 min** | ▪ Mount glass coverslips to microscope glass slide<br>▪ Cure samples overnight at RT (protected from light)<br>▪ View under microscope<br>▫ DAPI for nuclei: EX 387/11 nm, EM 447/60 nm (recommended Semrock P/N DAPI-11060B)<br>▫ ALEXA 488 for mRNA: EX 485/20 nm, EM 524/24 nm (recommended Semrock P/N FITC-2024B)<br>▫ Fast Red for miRNA: EX 530/40 nm, EM 590/40 nm (recommended Semrock P/N TRITC-B)<br>▫ ALEXA 750 for mRNA: EX 708/75 nm, EM 809/81 nm (recommended Semrock P/N Cy7-B) |

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