RNAscope™ Assay on Whole Zebrafish Embryos

This protocol is intended as a guide only, for full experimental details please read the product user manual.

Introduction

This Technical Note provides guidelines on how to run the RNAscope™ Assay on whole-mount zebrafish embryos.

Read the Safety Data Sheet (SDS) available on the website, and follow handling instructions. Wear appropriate protective eyewear, clothing, and gloves. For the latest services and support information, contact our Technical Services.

Part 1: Sample Preparation

Fix the Embryos

1. Collect zebrafish embryos at the desired developmental stages, and remove the chorions.
2. Place the embryos into one or more wells of a 24- well plate. Make sure each well contains a mesh insert (Ted Pella, Prod. No. 36173) before adding the embryos.
3. Fix the embryos using 3 mL per well of fresh 10% NBF at ROOM TEMPERATURE (RT) for 17–24 HRS.
4. Remove the fixative solution. Wash the embryos using 3 mL per well of 1X PBS + 0.1% Tween 20 (PBST) at RT for 10 MIN.

Dehydrate and Store the Embryos

1. Transfer the mesh insert containing the embryos to a fresh well containing 3 mL of 25% methanol in 1X PBST. Incubate at RT for 10 MIN.
2. Transfer the mesh insert containing the embryos to a fresh well containing 3 mL of 50% methanol in 1X PBST. Incubate at RT for 10 MIN.
3. Transfer the mesh insert containing the embryos to a fresh well containing 3 mL of 75% methanol in 1X PBST. Incubate at RT for 10 MIN.
4. Transfer the mesh insert containing the embryos to a fresh well containing 3 mL of 100% methanol. Incubate at RT for 10 MIN.

Part 2: Sample Pretreatment

Rehydrate and Permeabilize the Embryos

1. Transfer the desired number of embryos into one or more wells of a fresh 24-well plate. Make sure each well contains a mesh insert before adding the embryos.
2. Transfer the mesh insert containing the embryos to a fresh well containing 3 mL of 0.2 M HCl in 100% methanol. Incubate at RT for 30 MIN.
3. Transfer the insert containing the embryos to a fresh well with 3 mL of 75% methanol in 1X PBST. Incubate at RT for 10 MIN.
4. Transfer the insert containing the embryos to a fresh well with 3 mL of 50% methanol in 1X PBST. Incubate at RT for 10 MIN.
5. Transfer the insert containing the embryos to a fresh well with 3 mL of 25% methanol in 1X PBST. Incubate at RT for 10 MIN.
6. Transfer the insert containing the embryos to a fresh well with 3 mL of PBST + 1% BSA. Incubate at RT for 10 MIN.

NOTE: Transfer the embryos in 100% methanol to avoid the embryos sticking to the transfer pipette and mesh insert.

Apply RNAscope™ Target Retrieval

1. Add 200 mL of fresh 1X Target Retrieval solution to a Tissue-Tek® container.
2. Place the container in a steamer and heat the solution to 100°C.
3. Carefully transfer the insert containing the embryos into the heated 1X Target Retrieval solution. Incubate at 100°C for 15 MIN.
4. *Immediately* transfer the embryos to a fresh well containing 3 mL PBST + 1% BSA in a 24-well plate. Incubate for 1 MIN.
5. Transfer the insert containing the embryos to a fresh well, and wash with 100% methanol for 1 MIN.
6. Transfer the embryos into 1.5 mL microcentrifuge tubes, and carefully remove the 100% methanol.
7. Wash the embryos carefully by *slowly* adding 1 mL PBST + 1% BSA one drop at a time.

   IMPORTANT: The embryos may stick to the side of the tube when PBST + 1% BSA is added. If this occurs, replace PBST + 1% BSA with 100% methanol and repeat steps 6 and 7.

Apply RNAscope™ Protease Plus

1. Carefully remove as much of the PBST + 1% BSA wash as possible without letting the embryos dry.
2. Add 300 µL of Protease Plus, and incubate at 40°C for 5–60 MIN depending on the age of the embryos (5–15 minutes for 24 hpf, 30 minutes for 48 hpf, and 60 minutes for 72 and 96 hpf). Float the tube horizontally in a water bath.
3. Replace Protease Plus with 300 µL of Probe Diluent.
4. Remove Probe Diluent before continuing with the assay.

Part 3: RNAscope™ Assay

Probe Hybridization and Staining

Continue with the RNAscope™ 2.5 HD or V2 fluorescent assay steps from target probe hybridization to counterstaining using the appropriate Part 2 Detection User Manual available in the document download section on the product page.

Make the following modifications:

- Perform all hybridization steps in a 40°C water bath by floating the tubes horizontally.
- Perform all wash steps twice using 1X Wash Buffer for 10 MIN each time.
- **OPTIONAL:** At the end of the RNAscope™ assay, clear the embryos with CUBIC reagent by removing the wash buffer and adding 200 µL of CUBIC reagent (see below). Store at RT.

CUBIC Clearing Reagent: 10 g Triton X-100, 5 g

N,N,N′,N′-Tetrakis Ethylenediamine (Sigma, Cat. No. 122262), 10 g Urea, 80 µL of 3 M NaCl and add water to 100 g total.

Obtaining Support

For the latest services and support information, contact our Technical Services.

At the website, you can:

- Access telephone and fax numbers to contact Technical Support and Sales
- Search through FAQs
- Submit a question directly to Technical Services

Scan the QR code to review online

10991808009_0126