RNAscope® Assay on Whole Zebrafish Embryos

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, go to: **www.acdbio.com/support.**

Workflow

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**.

NOTE: Store the embryos in 100% methanol at **–20°C** for up to two months. Make sure the embryos do not dry out.

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.

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

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**.

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 at **http://www.acdbio.com/technical-support/user-manuals**.

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, go to: **https://acdbio.com/technical-support/support-overview**.

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 Support

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