Solution recipes for ISHpalette experiments

● Hybridization buffer (for 30 mL)

01. Mix the following reagents in a 30-mL tube:
    - Ultrapure water: approx. 15 mL
    - 20× SSC: 1.5 mL
02. Warm in a microwave to ~60 °C, avoiding bumping.
03. Once sufficiently warmed, add 3 g of Dextran sulfate and mix thoroughly.
04. When completely dissolved, add the following:
    - 10% Tween 20: 0.3 mL (final 0.1%)
    - 50 mg/mL Heparin: 30 µL (final 50 µg/mL)
    - 50× Denhardt's: 0.6 mL (final 1×)
05. Bring up the volume to 30 mL with ultrapure water and mix well.
06. Store at −20 °C.

● Amplification buffer (for 100 mL)

01. Mix the following reagents in a 100-mL tube:
    Reagent: Required amount (final concentration)
    - 20× SSC: 40 mL (8×)
    - 1 M $MgCl_2$: 10 mL (100 mM)
    - Ultrapure water: ~30 mL
02. Warm in a microwave to ~60 °C.
03. Add Dextran sulfate: 10 g (10%) and dissolve by incubating at 37 °C overnight.
04. Once Dextran sulfate is dissolved, add 20% Triton: 1 mL (final 0.2%).
05. Bring up the volume to 100 mL with ultrapure water.
06. Aliquot into tubes. Store working aliquots at 4 °C and stock at −20 °C. Before use, return the 4 °C aliquots to room temperature for ~1 h and vortex thoroughly.

● 0.5× SSCT (for 100 mL)

01. Mix the following reagents:
    - 20× SSC: 2.5 mL (final 0.5×)
    - 10% Tween 20: 1 mL (final 0.1%)
02. Bring up the volume to 100 mL with ultrapure water.
03. Store at room temperature.

Other reagents (if not specified, no particular requirement)

Heparin Sodium (FUJIFILM Wako) #081-00136 (other manufacturers acceptable)

Sodium Dextran sulfate 500,000 (FUJIFILM Wako) #193-09981 (lower MW less effective)

- The above information is subject to change without notice for product improvements, specification updates, or enhancements.
- This product does not cover or guarantee the performance or use of other companies' products.
- This development product has been co-developed and commercialized in collaboration with Toho University, utilizing the technology (Patent No. 7482506) developed by Associate Professor Yousuke Tsuneoka, Department of Anatomy (Division of Microscopic and Morphological Anatomy), Faculty of Medicine, Toho University.