DAICEL arbor biosciences

myTags® CUSTOM DESIGNED FISH PROBES

*This protocol is provided as a guideline and may need to be optimized by the researcher*

# myTags® Probes to Detect RNA in Adherent Cultured Cells

## Cell Fixation and Prehybridization:

All solutions for this stage and the RNA FISH Hybridization are prepared with RNase-free diH20 and RNase-free reagents. Instruments and supplies which could not be autoclaved or were not certified as RNase-free were treated with RnaseAway.

## Cell Harvesting, Fixation and Preservation:

Cells are rinsed briefly with 1xPBS then fixed with 4% formaldehyde in PBS prepared from 16% PFA stock supplied in sealed glass ampoules.

After fixation, washed three times in 1xPBS at five minutes per wash. The cells are then stepwise permeabilized for 3 minutes in 25% cold ethanol-PBS, 50% cold ethanol-PBS and 1 hour in 70% cold ethanol.

- Cells should be kept in the 70% cold ethanol for at least one hour.
- The cells can be stored in 70% ethanol at 4°C in tightly sealed culture plates for a month or more if necessary.
  - Best if used as soon as possible

## RNA FISH Protocol:

RNase-free environment is of high importance to maintain the RNA for probe binding and imaging.
All reagents, solutions and labware should be dedicated strictly for RNA work.

Hybridization Buffer (HB):

- 1g dextran sulfate,
- 1ml 20XSSC,
- 1ml deionized formamide, add RNase-free diH2O to 10ml total.

Wash Buffer (WB):

- 5ml 20xSSC,
- 5ml deionized formamide, and
- 40ml RNase-free di-H2O.

Avoid drying of cells throughout all steps.

Aspirate the 70% ethanol out of the well containing the cells, add appropriate vol. of WB and incubate for 2-5 minutes at room temperature.

Add 35 µL of the HB/probe mixture, with the total concentration of probe being 125nM. (scale the vol appropriate for culture wells)

- Titering of the amount of probe for your particular cell line may be required.
- Some protocols start with 20 ng probe per hybridization reaction

Cover the plate with the tissue culture lid, and seal with Parafilm, followed by aluminum foil or other light-tight material.

- Incubate in the dark at 37 °C for 4-16 hours.

Remove the hybridization mix wash with appropriate vol of WB.

- Incubate in the dark at 37 °C for 30 minutes, aspirate the WB,
- If part of experimental protocol proceed with ICC.
- Counterstain with Hoechst add anti-fade media (ProLong Diamond is recommended) and image.