



***t*DenHyb™ Solutions (for Tissue FISH on paraffin-embedded tissue slides)**

*t*DenHyb™ solutions are highly effective hybridization solutions for FISH on paraffin-embedded cells and tissue sections. *t*DenHyb™ solutions are compatible with a wide range of home-brewed or commercially available directly- and indirectly labeled DNA probes: repeat sequence, paint, and unique sequence probes.

- *t*DenHyb-1 is optimized for FISH on paraffin-embedded tissue sections when repeat sequence probes (*e.g.*, Vysis CEP probes) were used. But *t*DenHyb-1 is less effective for unique sequence probes.
- *t*DenHyb-2 is optimized for FISH on paraffin-embedded tissue sections when unique sequence probes (*e.g.*, Vysis LSI probes) were used. But *t*DenHyb-2 is less effective for repeat sequence probes.

Dilute or suspend labeled DNA probes in an appropriate *t*DenHyb™. Then, perform your in-house tissue FISH procedure with the DNA probes (in *t*DenHyb™). More effective and convenient FISH can be achieved by using Insitu MetalTray FISH protocols* which are based on the use of [Metal Slide Tray](#) and [HybBox™](#) in conjunction with the use of *t*DenHyb.

If you perform FISH with Insitu *t*DenHyb™ solutions using your in-house FISH protocol or protocol provided by vendors that sell DNA probes, a slight adjustment of your denaturation condition close to the Insitu Manual Tissue FISH protocols would maximize hybridization signals.

If you are doing FISH with your home-brewed DNA probes, simply dilute or suspend your probes in *t*DenHyb™ solution. Add blocking DNA if necessary. The optimal concentration of the home-brew probe must be determined empirically.

If you are using commercial repeat sequence probes (*e.g.*, Vysis), you may dilute these probes by **100- to 500-fold**, depending on probes, with *t*DenHyb-1 solution. Hybridization in HybBox™ or other system for 2-3 hours is sufficient to view good signals.

If you are using commercial unique sequence probes (*e.g.*, Vysis), you may dilute these probes by **50- to 100-fold**, depending on probes, with *t*DenHyb-2 solution. For ready-to-use **Vysis PathVysion HER-2 DNA Probe Kit**, the premixed PathVysion probes can be diluted at 1:5 or 1:10 with *t*DenHyb-2. Overnight hybridization in HybBox at ambient temperature or humid box at 37°C is recommended to increase signal intensity. For mixed probes containing unique sequence and repeat sequence probes (as a reference probe), dilute both types of probes in *t*DenHyb-2.

***t*DenHyb solutions can be used for the application to tissue microarrays (TMA) with DNA probes.**

Available *t*DenHyb* are:

- Catalog # INS-D101; *t*DenHyb-1, 1 ml
- Catalog # INS-D102; *t*DenHyb-2, 1 ml

*Both *t*DenHyb-1 and *t*DenHyb-2 are very turbid.