3. *t*DenHyb for Tissue Microarrays (TMA):

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Recommended DenHyb Solution and Dilution for Commercially Available Probes*

A. Vysis Directly-Labeled Probes

Probe	Recommended DenHyb	Recommended Dilution**
Repetitive Probes		
Interphase Nuclei	*c*DenHyb-1	200 - 500 fold
Metaphase Spreads	*c*DenHyb-1	100 - 250 fold
Paraffin-Embedded Tissue Sections	*t*DenHyb-1	200 - 500 fold
Unique Sequence Probes		
Interphase Nuclei	*c*DenHyb-2	100 - 150 fold
Metaphase Spreads	*c*DenHyb-2	50 - 100 fold
Paraffin-Embedded Tissue Sections***	*t*DenHyb-2	50 - 150 fold
Painting Probes		
Metaphase Spreads	*c*DenHyb-1	100 fold

B. Oncor's (defunct) Digoxigenin-Labeled Probes

Probe	Recommended DenHyb	Recommended Dilution**
Repetitive Probes		
Interphase Nuclei	*c*DenHyb-1	20 - 40 fold
Metaphase Spreads	*c*DenHyb-1	10 - 20 fold
Paraffin-Embedded Tissue Sections	*t*DenHyb-1	10 - 20 fold
Unique Sequence Probes		
Interphase Nuclei	*c*DenHyb-2	10 fold
Metaphase Spreads	*c*DenHyb-2	10 fold
Paraffin-Embedded Tissue Sections	*t*DenHyb-2	5 - 10 fold
Painting Probes		
Metaphase Spreads	*c*DenHyb-1	10 - 20 fold

* Dilution for paraffin-embedded tissue sections was based on the use of xylene-based deparaffinization.
** Dilution in *c*DenHyb or *t*DenHyb solution.
*** Vysis pre-diluted, ready-to-use PathVysion probes can be diluted by 1:10 dilution in *t*DenDenHyb-2.

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DenHyb Hybridization Solutions for FISH

Two types of DenHyb solutions:

a) *c*DenHyb (D001 and D002) for cell FISH

- ***c*DenHyb-1** for repeat sequence or paint probes
- ***c*DenHyb-2** for unique sequence probes

b) *t*DenHyb (D101 and D102) for tissue FISH

- ***t*DenHyb-1** for repeat sequence probes
- ***t*DenHyb-2** for unique sequence probes

DenHyb solutions are:

- Very effective hybridization solutions for cell and tissue FISH
- Compatible with a wide range of home-brewed or commercially available DNA probes
- Compatible with most of FISH protocols
- Stable almost indefinitely if they were kept stored at -20°C

Advantages to using DenHyb solutions:

- Reduce the cost of expensive commercial probes for FISH
- Save precious home-brewed probes for FISH
- Save time and effort from preparing your own hybridization solutions

Storage: Store frozen at -20°C until use.

Rev. Date: 4/17/04 Catalog No. 02-110f

Method of use:

a) Dilution or suspension of Commercial DNA probes: Dilute or suspend DNA probes in an appropriate *c*DenHyb or *t*DenHyb solution:

200 - 500 fold dilution for Vysis CEP probes
50 - 150 fold dilution for Vysis LSI or paint probes
5 - 10 fold dilution for ready-to-use pre-diluted probes (*e.g.*, PathVysion)

b) Dilution or suspension of home-brewed DNA probes: Dilute or suspend home-brewed probe in an appropriate *c*DenHyb or *t*DenHyb solution. If it is necessary, add blocking DNA. The optimal concentration of the home-brewed probes must be determined empirically.

** For dilution/suspension of probes, avoid including water or hybridization solutions other than Insitus DenHyb solution.*

c) Selection of *c*DenHyb solutions and probes for cell FISH

- **Repeat sequence (satellite, centromeric) and paint probes:** Dilute or suspend probes in *c*DenHyb-1 solution (Cat. # D001).
- **Unique sequence (locus specific) probes**: Dilute or suspend probes in *c*DenHyb-2 solution (Cat. # D002).
- **Mixed probes containing unique sequence and repeat sequence probes**: Dilute both types of probes in *c*DenHyb-2 solution (Cat. # D002). Alternatively, repeat and unique sequence probes can be diluted in *c*DenHyb-1 and *c*DenHyb-2, respectively, and then apply separately.

d) Selection of *t*DenHyb solutions and probes for tissue FISH

- **Repeat sequence (satellite, centromeric) probes**: Dilute or suspend probes in *t*DenHyb-1 solution (Cat. # D101).
- **Unique sequence probes (locus specific probes):** Dilute or suspend probes in *t*DenHyb-2 solution (Cat. # D102).
- **Mixed probes containing unique sequence and repeat sequence probes:** Dilute both types of probes in *t*DenHyb-2 solution (Cat. # D102). Alternatively, repeat and unique sequence probes can be diluted in *t*DenHyb-1 and *t*DenHyb-2, respectively, and then apply separately.

e) General FISH Protocols for DenHyb-containing probes:

FISH with diluted/suspended DNA probes (in *c*DenHyb or *t*DenHyb) can be performed according to any of the following protocols:

i) Your proven, in-house protocol: Follow the protocol and apply the probes which were diluted or suspended in DenHyb solution.

ii) Protocols provided by vendors that sell DNA probes: Follow the protocol and apply the probes which were diluted or suspended in DenHyb solution.

iii) Insitus Manual Protocols: These protocols are optimized for DNA probes which were suspended or diluted in DenHyb solution and are available upon request. Please see below **f) Available Insitus FISH protocols**.

iv) Insitus MetalTray FISH Protocols: These protocols are based on the use of Metal SlideTray, HybBox, and DNA probes (in DenHyb solution), and are available upon request. Please see below **f) Available Insitus FISH protocols**.

f) Available Insitus FISH protocols: Following protocols are available upon request :

2-Well Slide FISH (protocol catalog # 01-016f)
Manual Cell FISH (protocol catalog # 01-011p)
Metal Tray Cell FISH (protocol catalog #. 01-012p)
SkipDewax as a Binfunctional Pretreatment Solution (protocol catalog # 02-025f)
Tissue Pre-Conditioner as a Pretreatment Solution (protocol catalog # 01-027f)
Manual Tissue FISH (protocol catalog # 01-021p)
Metal Tray Tissue FISH (protocol catalog # 01-022p)
Complete Pre-Treatment Protocol for Formalin-Fixed, Paraffin-Embedded Tissue Sections with Xylene and Pretreatment Solution (Protocol Catalog #. 01-020p)
Complete Pre-Treatment Protocol for Paraffin-Embedded Tissue Sections with SkipDewax (protocol catalog # No. 01-024p)

Citations

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