



How to Use ONCYTE[®] Film-Wells / Film-Slides Instructions for Immunohistochemical Staining

1. Apply specimen to Film area (see side A).
2. Air dry
3. Fix specimens using appropriate fixative for the antigen studied. Commonly recommended fixatives are:
 - A. Paraformaldehyde: 4% in phosphate buffered saline (PBS). 0.05M magnesium chloride, 0.3% Tween-20, pH 7.4. Fix at 4°C. Fixation time may vary according to specimen thickness and specific antigen.
 - B. Zamboni's: *Nature* 216:174-175, 1967.
4. Wash with three changes of PBS containing Tween-20 (0.1%) pH 7.5 (TPBS).
5. Block non-specific binding of primary antibody:
 - peroxidase based detection: incubate specimens for 15 minutes with 0.3% hydrogen peroxide prepared in de-ionized water.
 - alkaline phosphatase based detection: incubate specimens 15 minutes at room temperature in TPBS containing 1% normal serum from the species in which the secondary antibody is made.
6. Blot excess serum from specimens.
7. Incubate specimens for 30 minutes with primary antibody diluted in TPBS containing 1% serum.
8. Wash Film-slides with 3 changes of TPBS.
9. Incubate with secondary antibody diluted in TPBS containing 1% normal serum.
10. Repeat Step 8.

Note: remaining steps refer to alkaline phosphatase based detection.

11. Incubate specimens in alkaline phosphatase labeled reagent.
12. Repeat Step 8.
13. Equilibrate specimens for 2 minutes in 0.1M TRIS pH 9.5.
14. Incubate in substrate or solution 20-30 minutes.
15. Rinse with tap water.
16. Counterstain if desired (see side A).
17. Air dry or dehydrate through isopropyl alcohol series.
18. Clear with 3 changes of xylenes.
19. Coverslip using non-aqueous mounting medium.

OnLine Technical Information and Assistance: www.gracebio.com

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How to Use ONCYTE[®] Film-Wells / Film-Slides

- Specimens may be applied to a Film-Slide by any conventional method including centrifugation, cryostat mounting, tissue printing pipetting, and array depositors.
- Film-Slides provide superior specimen adherence, out performing silane and protein treated glass slides.
- Soluble analyte may be bound to Film-slides by air drying, UV crosslinking, baking or microwaving. Film-slides are autoclavable.
- Specimens may be chemically fixed using aqueous fixatives or compatible alcohols.
- Film-slides are ideally suited for use in most cytochemistry assay protocols including ICC, ISH, ISPCR and HTS.
- Film-slides are chemically resistant to reagents typically used in most assays for cell analysis including 50% formamide.
- Film-slides are not recommended for use with acetone, ethanol & methanol. Isopropanol and butanol are common substitutes.
- Bi-directional access to specimen by reagents may reduce incubation times and reagent concentrations.
- Film-slides are compatible with a variety of counterstains including Mayer's hematoxylin, nuclear fast red and light green SF. Gill's hematoxylin is recommended (0.05% HCl may be used to de-stain). Eoin (0.5%, diluted in isopropanol). In some cases counterstains may bind to the film, this usually does not interfere with the microscopic examination of specimens.
- Film-Slides are made transparent for microscopic and imaging applications by xylenes or immersion oil with a refractive index of 1.515. For aqueous mounting Vectashield (Vector Labs), Slow Fade & Slow Fade Light (Molecular Probes, Inc.) are recommended.
- Permanent sections may be made by coverslip mounting specimens using non-aqueous media after dehydration through alcohol or by air drying.

REFERENCES:

High-Definition Cell Analysis *In Situ* Using Microporous Films.
Cel Vision 2:499-509, 1995.

Cytometrically Coherent Transfer of Receptor Proteins on Microporous Membranes.
BioTechniques 20:641-650. 1991.

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ONCYTE Film-Slides & Film-Wells are Intended for Laboratory Use Only