KIDDRC
Histology Services
Protocols

For more info contact: Jing Huang, Histology Specialist
913-588-5996

Basic Immunocytochemistry procedure (lab method)

1. Deparaffinize slides and run down through graded alcohol to dHOH.
For frozen sections: Let slides come up to room temperature and air dry.
If fixed, hydrate slides in 3 changes of dHOH.
If unfixed, fix in cold acetone for 10 minutes.
Let slides air dry. Rehydrate with three changes of dHOH. If using another fixative, fix slides for 10-3- minutes, and wash in three changes of dHOH.

2. 3% HOOH for 15 minutes.

3. Rinse twice with DHOH, then once with TBS.

4. Incubate slides with 10% Normal Goat Serum (NGS) for 20 minutes.

5. Incubate slides with primary antibody for appropriate time.
overnight at 4°C
2hrs. at room temp.
1hr. at room temp. or 37°C

6. Rinse 3 with TBS (Rinse X3 quick, then 2-5 minutes washes.)

7. Incubate with biotinylated GAR or GAM 1:200 for 30 min. at room temp.

8. Rinse X 3 with TBS.

9. Incubate with HRP-avidin D (or streptavidin D) 1:1000 for 10 min. at room temp.

10. Rinse X2 with TBS. Rinse X1 with distilled water (not deionizeddeionized water can inhibit DAB)

11. Develop slides---approx. 5min. at room temp. Observe reaction under microscope.
DAB-Zymed Kit: 1ml distilled water
1 drop buffer concentrate rgnt. (bottle 1)---mix
1 drop rgtn 2 (DAB chromogen 20 X)--mix
1 drop rgnt 3 (HOOH)---mix

After developing, rinse in 3 changes of distilled water, then use DAB enhancer, if desired. Add 1 drop of rgnt. 4 to 1 ml of distilled water and mix.
Cover tissue and incubate 1-3 minutes at room temp. Color development may be monitored under scope.
Rinse in 2-3 changes of water.
DAB-Vector Kit: 2.5 ml. distilled water
1 drop buffer sol'n--mix
2 drops DAB, mix
1 drop HOOH, mix
(can add 1 drop nickel sol'n for black chromogen)

After development, rinse, then incubate slides in copland jar with 1% Copper Sulfate for 5 minutes. Rinse and procede.
True Blue (Kirkegard-Perry): Let come to Room temp, use full strength.
* Primary Ab should be at a higher dilution (lower concentration) than with DAB.

12. Dehydrate slides to xylene and coverslip with a nonaqueous mountant.

To Use Alkaline Phosphatase:
1. After secondary Ab, use Alkaline Phosphatase-avidin D as enzyme 1:50 for 20 min. at room temp.

2. Rinse X3 with TBS.

3. Develop with Vector Red: usually about 10-20 minutes at room temp.
2.5 ml 100 mM Tris HCL pH 8.2
1 drop Rgt. 1, mix
1 drop Rgt.2, mix
1 drip Rgt.3, mix
1 drop levamisole
*Vector Red is also fluorescent with rhodamine, fluorescein or AMCA excitation filter.

4. Dehydrate to xylene and coverslip.

TBS(TRIS BUFFER SALINE---0.5M)
Stock solution .5M
9.875g Tris Base
65.94g Tris HCL
fill to 1 liter with distilled water
80g NaCl
pH to 7.6

Working solution
100ml stock TBS
900ml distilled water

Dilution Buffer
8.640 ml TBS
960 µl Normal goat serum
400 µl 1% BSA
10 µl .02% sodium azide
use this buffer for diluting the primary antibody

Sodium azide: for 25 ml of .02%
0.005g/25ml

100mM Tris HCl (for alkaline phosphatase)
1.32g Tris Base
2.215g Tris HCl
250 ml d water
pH to 8.2