

# ICC-APAAP

## For Acetone-Methanol Fixed Slides both Tissue and Cytospin.

Retrieve slides from freezer and defrost in foil wrapper. Score around tissue on the back with diamond pen. Circle tissue with PAPPEN and write AB on slide.

1. Incubate each section with 1-2 drops of *Universal Blocking Solution* for 15 min.. Drain excess blocker and immediately apply primary Ab. as in step 2. **Do not wash slide.**
  2. Incubate each section with  $\pm 50 \mu\text{l}$  of the **primary AB** (i.e. CD3-Mse x Hu@1:100) diluted in **Antibody Diluting Buffer [ADB]** in a humid chamber overnight at 4C in the fridge; (this amount of antibody is appropriate for small biopsy specimens or cytopsin samples). If you have large sections you must apply more, tissue must be completely covered.
  3. Wash in TBS for 2- 5 minutes.(twice)
  4. Wipe carefully around each section, **DO NOT ALLOW THE SECTIONS TO DRY.** Add **2° antibody** (i.e.Rb x Mse-Ig (RAM )@ 1:60) **diluted in ADB.** Incubate at room temperature for 30-minutes.
  5. Wash in fresh TBS for 2- 5 minutes.(twice)
  6. Wipe around sections, then incubate with Mouse APAAP @ 1:60 diluted in **ADB** for 30 minutes as above.
  7. Wash in TBS for 2-5 minutes. (twice)
- Note:** After this step you can either go to the Fast Red, or do an enhancement by repeating step 4,5 6,7, if necessary.
8. Develop with Fast Red dissolved in the alkaline phosphatase substrate. Filter through  $0.2\mu\text{m}$  filter prior to use. Prepare 10 ml for about 20 slides. Incubate for 20 minutes **Maximum.** **FOR AN UNKNOWN ANTIBODY:** check slides under microscope as they develop. When positive, stop further reactions (to prevent high background) by immersing in TBS.
  9. Wash in TBS for 5 minutes then in water for 5 minutes.
  10. Counterstain in Gill II Haematoxylin for 10-15 seconds, depending on the age of the stain. **(NOTE: DO NOT USE ALCOHOL AS FAST RED IS SOLUABLE IN ALCOHOL).**
  11. Wash until water is clean. Place in Lithium carbonate for 20 seconds. Rinse well in tap water.
  12. Wipe bottom with a tissue. Place a very thin layer of **Crystal Mount** over the tissue.
  13. Dry the slides in the oven at 37 C overnight (or at 60 C for 1 hour.)
  14. The next day, you can coverslip over the Crystal Mount with a permanent mountant and coverglass. Use a mounting medium such as Cytoseal 60 (Fisher),

**IMMUNOCYTOCHEMISTRY PROTOCOL**  
**APAAP-POLYCLONAL AB**

*Works best on paraformaldehyde-fixed slides.*

**CYTOSPINS** using GRB-1 polyclonal ab. - > J.R. Leung

1. Hydrate slides in TBS      **5 min. at R.T.**
2. Block non-specific binding with 'universal' blocking sol. **15 min. at R.T.**
3. Drain slides (but do not wash ) and incubate with GRB-1 -dil. 1:100 in Antibody Diluent. **Overnight at 4 C.**
4. Next day: Wash slides in TBS **5 min. at R.T.**
5. **2nd AB:** Swine anti-rabbit IgG-Biotinylated - dil. 1:30 for **30 min. at 37 C.**
6. Wash in TBS **5 min. at R.T.**
7. Avidin -Alkaline phosphatase - dil. 1:30 -for **30 min. at 37 C.**
8. Wash in TBS **5 min. at R.T.**
9. **If you need to enhance the immunoreactivity -repeat step 5 through step 8**
10. Developing stain :  
Fast Redsubstrate ( see APAAP #1 for remaining steps )

**NOTE:**

Some of the protocols, use Human serum to block non-specific binding. Which is perfectly ok to use. Make sure your 2nd Ab and Apaap are diluted in TBS with 20 % Human serum.

Universal blocking sol. and Antibody diluent are maybe more expensive but it does give uniform results. And we have switched over to this.

**IMMUNOCYTOCHEMISTRY PROTOCOL**  
**APAAP-POLYCLONAL AB**

*Works best on paraformaldehyde-fixed slides.*

**CYTOSPINS** using GRB-1 polyclonal ab. - > J.R. Leung

1. Hydrate slides in TBS      **5 min. at R.T.**
2. Block non-specific binding with 'universal' blocking sol. **15 min. at R.T.**
3. Drain slides (but do not wash ) and incubate with GRB-1 -dil. 1:100 in Antibody Diluent. **Overnight at 4 C.**
4. **Next day:** Wash slides in TBS **5 min. at R.T.**
5. **2nd AB:** Swine anti-rabbit IgG-Biotinylated - dil. 1:30 for **30 min. at 37 C.**
6. Wash in TBS **5 min. at R.T.**
7. Avidin -Alkaline phosphatase - dil. 1:30 -for **30 min. at 37 C.**
8. Wash in TBS **5 min. at R.T.**
9. **If you need to enhance the immunoreactivity -repeat step 5 through step 8**
10. Developing stain :  
Fast Red substrate ( see APAAP #1 for remaining steps )

**NOTE:**

Some of the protocols, use Human serum to block non-specific binding. Which is perfectly ok to use. Make sure your 2nd Ab and Apaap are diluted in TBS with 20 % Human serum.

Universal blocking sol. and Antibody diluent are maybe more expensive but it does give uniform results. And we have switched over to this.

**IMMUNOCYTOCHEMISTRY PROTOCOL**  
**APAAP - POLYCLONAL AB**

*All incubations at R.T. unless stated otherwise stated*

*Rat lung Paraformaldehyde fixed*

1. Permeabilize with 0.2% Triton X-100 for **20 minutes**. Wash in TBS.
2. Blocking: Milkbovine 3% in distilled water for **1 hour**. Wash in TBS.
3. 1st AB Polyclonal AB  $\alpha$  Glucocorticoid R. PA1-511  
Dilute at 1:500 in TBS  
Overnight at 4° C (**20 Hours**).  
Next day: Wash in TBS
4. 2nd AB Swine  $\alpha$  Rb IgG-Biotin Dilution 1:30 in TBS for **1 hour**. Wash in TBS.
5. Avidin - AP dilution 1:30 in TBS for **1 hour**. Wash in TBS.
6. Developing Stain: Fast Red   
Substrate  1 mg/ml from 0-20 minutes. Check under microscope.
7. Wash in TBS.  
Wash in H<sub>2</sub>O.  
Counterstain.  
Crystal Mount.  
Dry and place coverslip.

Reference: D. Bellingham et al  
Mol. Endo 6:2090-2102 (1992)