

PROTOCOL OF IMMUNOSTAINING FOR PARAFFIN SECTION

by JYP 8/13/02

DeWax

1. Use EZ-DeWax Solution X 4 minutes*.
2. Incubate slides in Xylene X 30 minutes.

Rehydration

1. Incubate slides in 100%→70%→50% ETOH X 5 minutes each.

Antigen Retrieval

1. Rinse slide well in distilled water; place slide in plastic holder with empty slots filled with blank slides.
2. Place the holder in white bath with Antigen Retrieval Solution covering sections and place lid loosely on.
3. Turn the oven on high power, watch the solution until it comes to **rapid boil**, and turn off the oven.
4. Set oven (SHARP, carousel) power to 20% level and heat for 10-15 minutes (the power setting should be adjusted so that the oven cycles on and off every 20-30 seconds and the solution boils about every 5-10 seconds each cycle).
5. Remove container from oven, allow it to cool for 30 minutes.
6. Wash with PBS, 2 minutes X 2.
7. Circle the section with Pap-pen.
8. Place slide in a humidity chamber (by placing wet paper under the slide) with section covered by PBS.

Primary and Secondary antibody incubation

1. Cover the section with the primary antibody (diluted in goat serum: 1:250) X 1-3 hr. @ RT or O.N.
2. Wash with PBS, 2 minutes X 3.
3. Add 6 drops (or simply enough drops to cover the section well) of "Link"*.
4. Incubate 20 minutes @ RT.
5. Remove antibody and wash with PBS for 20 minutes, changing PBS a total of 4 times during 20 minutes.
6. Add 6 drops (or simply enough drops to cover the section well) of "label"*, incubate @ RT. X 20minutes.
7. Remove "Label" and wash with PBS for 20 minutes, changing PBS a total of 4 times during 20 minutes.

DAB staining

1. Cover the section with DAB mixture* (about 600 μ l, or as much as you can add to the section well) and monitor the color develop (need about 8min to turn to dark brown).
2. Wash with PBS X 3 minutes X 2.
3. Counterstain with Light Green working solution (about 600 μ l, or as much as you can add to the section well), monitor the color change (about 10min).
4. Dehydrate by alcohol: Cover the section with 95% ETOH X 5 minutes, remove; Cover the section with 100% ETOH X 5 minutes, remove; then Xylene X 5 minutes X 2, remove
5. Mount the slide with permount, cover the section with cover slip

* EZ-DeWax Solution: made by BioGenex, HK585-5K

* MULTILINK: made by BioGenex, HK340-5K (color yellow)

* LABEL: made by BioGenex, HK330-5K (color red)

* 1X DAB mixture made by: dilute 10 X DAB/Metal Concentrate (made by PIERCE, CAS 60-11-7)
with 1 X Stable Peroxide Substrate Buffer (made by PIERCE, No.1855910)