Fos immunocytochemistry

Day 1

1. Prepare the **BUFFER**: PBS or TPBS with 1% NGS and 0.3% triton.

2. Add antibody (one 20 µl/tube of 1:20 dilution, in Revco freezer) to 0.5 ml of buffer in a glass tube.

3. Place 15-20 mg of rat liver powder[1] in the glass tube.

4. Incubate in the refrigerator overnight or put glass tube in 37^{o} C oven for 1 hour followed by 1 hour in 4^{o}C refrigerator. Agitate gently once or twice during this time.

5. Wash tissue + **control** twice in the buffer with agitation.

6. Remove liquid from wells.

7. Pre-incubate the sections, on the shaker, for at least 60 min in BLOCKING SOLUTION. DO NOT WASH AFTER BLOCKING.

8. Rinse glass tube containing the antibody-liver powder solution with 5 ml of buffer and transfer this solution (5.5 ml) in a Centrex centrifuge tube. Centrifuge at 3500 rpm for 10 min. Then add 14.5 ml of buffer (final volume =20 ml).

9. Empty the wells and apply 400 µl of primary antibody per well. Incubate for 48 hours on shaker table at 4^{o} C.

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[1] Cooper Biomedical Rat Liver Powder. Cat#0013-1140.

Day 2

1. Wash with buffer 3 times with agitation for 5-10 min between rinses.

2. Dilute secondary antibody (**bridge**): 1 drop of GAR per 10 ml of buffer.

3. Empty the wells and apply 400 µl of secondary antibody per well.
   Incubate for 60 min at room temperature.

4. Wash 3 times with **NEW** buffer: PBS or TPBS with 0.3% triton (NO SERUM).

5. Dilute standard ABC (Vector PK4000) 30 min. before using it: add 2 drops of solution A and then 2 drops of solution B per 10 ml of **new** buffer. Never mix A and B before adding the buffer. Never mix solutions from different kits.

6. Empty wells and apply 400 µl of ABC per well, for 60 min with agitation.

7. Wash three times with PBS or TPBS (no triton, no serum).

8. Refrigerate until DAB reaction.