TISSUE PROCESSING / SOLUTIONS FOR X-GAL & IMMUNO

**X-gal solution for cell cultures:**

**Reaction buffer (10 ml):**

To 8.27 ml 1x PBS add:

- 0.5 ml 0.4 M K-ferrocyanide (35mM)
- 0.2 ml 100 mM MgCl2 (2mM)
- 10 µl Sodium deoxycholate (0.01%)
- 20 µl NP40 (0.02%)

Add 0.5 ml X-gal stock (20 mg/ml) to reaction buffer (0.4 mg/ml)

pH 7.4 - 7.8 (could use pH 8.5 to inhibit endogenous ß-gal)

**HRP immunohistochemistry:**

Rinse PBS 1X

Quench in 3% H2O2 / 20% Methanol in PBS 20 min RT

Block 10% (goat) serum 30 min - 1hr

**Primary:** dilute in BSA/TX100/PBS 2hr RT or 4C overnight

Rinse 3x PBS 5 min each

**Secondary:** dilute in PBS 1/500 45 min - 1hr

Rinse 3x PBS 5 min each

**Avidin/HRP:** dilute in PBS 1/500 45 min - 1hr

Rinse 3x PBS 5 min each

**DAB** (3-5 mg per 10 ml PBS) - **ADD 10 µl per 10ml H2O2!!!** prior to use incubate no longer than 10 min

Rinse in PBS