

PAS Staining

(Periodic Acid Schiff Staining for Mucin)

(Sigma Cat# 375810)

0.25g dissolved in 50mL MilliQ water → 0.5% PA Solution

Schiff's Reagent (Sigma Cat# 3952016-500mL)

Light sensitive! Store at 4C, warm to room temp before use

Hematoxylin Solution (Gill No.3; Sigma Cat# GHS316-500mL)

Process Takes ~ 2 hours

1. Deparaffinize and hydrate paraffin embedded sections:

Histoclear 2x 5mins → 100%EtOH 2x 3mins → 95%EtOH 2x 3mins → 70%EtOH 2x mins → MQ Water 2x 3mins

2. Place slides in Coplin jar with 0.5% PA solution, 10mins, RT, bench top

3. Pour off PA solution (keep protected from light, 4C, can be re-used 4-6x)

4. Rinse slides in Coplin jar 3x in MQ water 1 min each

5. Add Schiff's Reagent for 15mins (with lid on), RT, bench top

6. Remove Schiff's (can keep and re-use 4-6x, store at 4C in plastic 50mL foil-covered tube)

7. Wash slides in running tap water (slightly warm), for 10 mins (can transfer slides to black rack and use bucket for tap water wash steps)

8. Counterstain slides by moving rack from water to Hematoxylin (Gill No.3) in bucket for 30sec (important! Be quick and have a fresh tap water filled bucket waiting to prevent over-staining)

(Pour Hematoxylin back into stock bottle for re-use – stable at RT for 1yr. protected from light)

9. Rinse slides in running (slightly warm) tap water for 5mins

10. Dehydrate, and clear → mount

70%EtOH 2x mins → 95%EtOH 2x 3mins → 100%EtOH 2x 3mins → Histoclear 2x 5mins → Permount mounting media; allow to dry 2hrs at RT before imaging