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Post-PhenoCycler H&E

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Protocol status: Working

We use this protocol and it's working

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Abstract

This procedure is for removing the glass flow cells and performing H&E staining following the completed PhenoCycler assay.

Flow Cell Removal

- 1 Place sample slides in a staining dish or coplin jar containing Histoclear. Allow to sit overnight at room temperature.
- 2 Remove the glass flow cells from the sample slides. Use a razor blade if necessary
- 3 Rinse the sample slides in at least 1L of DI water
- 4 Wash the sample slides in 1x PhenoCycler Buffer without additive, 3 × 10 minutes.
- 5 Proceed to H&E immediately or transfer slides to PhenoCycler Storage buffer and store 4°C

H&E Staining

- 6 Prepare the following solutions in coplin jars, staining dishes, or an automatic slide stainer. Immerse the tissue completely and agitate occasionally. Depending on the manufacturer, adjust the incubation times for the hematoxylin and eosin as needed. Xylene steps must be performed in a fume hood.

Filter hematoxylin and eosin with filter paper before use. Change out hematoxylin and eosin per manufacturer's guidelines, or every 2 weeks. Change out all other solutions every usage. If staining a high volume of slides, change out other solutions multiple times as needed.
- 7 MilliQ water, 2 minutes
- 8 Mayer's hematoxylin, 15 minutes
- 9 MilliQ water, 3 × 1 minute
- 10 Bluing solution, 1 minute



- 11 MilliQ water, 1 min
- 12 70% ethanol, 2 minutes
- 13 95% ethanol, 2 minutes
- 14 Eosin, 1 minute
- 15 95% ethanol, 2 × 30 seconds
- 16 100% ethanol, 2 × 30 seconds
- 17 Xylene, 2 × 3 minutes
- 18 Mount and coverslip. Allow to dry for at least 30 minutes room temperature.
- 19 Image in brightfield at 40X.