DNA Counterstain used by PSC

BRM and GD - 2/27/2019

NOTE – This protocol is appropriate for most IF slides and is required if images are obtained with the Aperio ScanScope FL. Read the reference documents carefully for more information.

Supplies Needed:

Reagent	Stock Concentration	Supplier	Reference
Hoechst 33258*	10 mg / mL	Molecular Probes #H3569	http://tools.lifetechnologies.com/content/sfs/manuals/mp21486.pdf
Deionized Water (dH_2O)		Purified in-house	
ProLong Gold Antifade Mounting Medium		Life Technologies #P36930	http://tools.lifetechnologies.com/content/sfs/manuals/mp36930.pdf

Procedure:

1. Prepare dilutions of Hoechst 33258. The stock solution is 10 mg/mL. Make a working solution of 100 µg/mL (1:100 dilution) in dH_2O and store in amber or light-resistant Eppendorf tubes at 4°C. Stock solutions in water are stable for six months when refrigerated.
2. At the time of staining, dilute the working solution (again 1:100 in dH_2O) for a final concentration of 1 µg/mL.
3. Add enough of the working solution to cover the tissue, then incubate at room temperature for 15 minutes.
4. Rinse slides three times (3x) in dH_2O, 5 minutes per rinse, using a light-resistant container.
5. Remove any excess water from around the tissue before mounting the coverslip. A folded Kimwipe works well for this purpose.
6. Add 1-3 drops of pre-warmed ProLong Gold mounting medium to the tissue. The quantity depends on the size of the tissue section. Then apply the coverslip†.
7. Incubate the slides in the dark for 24 hours at room temperature. NOTE – The refractive index increases with curing time.
8. Seal coverslip with Nail polish and let dry if prolonged storage is needed or thick (>5 um) sections are being prepared.
9. View the slides as usual.

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* Hoechst 33342 or DAPI can be substituted for Hoechst 33258 but may require a slightly different dilution. The laboratory should perform a titration experiment to determine the optimal concentration for representative specimens.

† TPL has found that coverslips often have a residue that appears as blue artifact under fluorescent microscopy. This residue can usually be removed by a quick rinse with deionized water on both sides of coverslip before mounting.