LANA1 Immunofluorescence Assay Protocol

Materials needed:

10x PBS
BSA (Sigma A-4378)
Anti-human IgG FITC
Anti-human IgM FITC

Solutions:

1x PBS-Tween
100mL 10x PBS
5mL 10% Tween

3% BSA in PBST
1.5g BSA
50mL 1x PBST
Sterile filter, store 4ºC

3% BSA in PBS
Same as for BSA-PBST, only with 1x PBS

Spotting slides:

1. Using BCP-1 cells with at least 70% viability, dilute in PBS such that each spot will receive 20000-25000 cells.
2. Use a cytopsin funnel and centrifuge to spot cells on a Probe-on slide.
3. Centrifuge 5 minutes at 1200RPM.
4. Allow slides to dry about 1 hour in the hood before fixing 20 minutes in paraformaldehyde.
5. After fixing, rinse twice in PBS by immersing the slides, do not let slides sit in PBS.
6. Gently pat slides dry and circle each spot with a hydrophobic Pap Pen.
7. Slides can be frozen at -80ºC.

Immunofluorescence:

1. Block each spot for 30 minutes with 50uL 3% BSA-PBST. Incubate in a slide box at room temperature with damp Kimwipes to humidify.
2. While slides are blocking, dilute sera 1:100 in BSA-PBS (1ul serum in 99ul BSA-PBS)
3. After blocking aspirate each spot. Be careful not to let spot dry out completely.
4. Add 50uL diluted serum to each respective spot and incubate 30 minutes at room temperature.
5. Aspirate the serum from each spot and wash the slides 5x for 3 minutes each with PBST in a coplin jar on the shaker.
6. Add 50 mL of secondary antibody to each spot and incubate 30 minutes at room temperature in the dark.
7. Aspirate each spot and wash 5x as before, but in the dark as the slides are now light-sensitive.
8. Add a small drop of VectaShield medium next to each spot so that a thin layer results when the coverslip is put on.