

Staining for Cytoplasmic IgM (μ) for Flow Cytometry

(Medina et al. (2000))

Reagents:

4% paraformaldehyde in PBS (pH=7.4)

4 ml 10% Neutral Buffered Formalin

6 ml PBS (pH=7.4)

PBS containing 0.2% Tween-20

9.98 ml PBS (pH=7.4)

20 μ l Tween-20 (Sigma #P2287)

Method:

1. Stain cells as usual for surface antigens.
2. Fix cells in 4% paraformaldehyde in PBS (pH=7.4) for 10 minutes @ 4°C.
3. Permeabilize cells by incubating in PBS containing 0.2% Tween-20 for 20 minutes at room temperature.
4. Incubate with FITC-labeled Goat antimouse IgM. Antibody.
 - o Fab'2 fragments work best.
 - o Use appropriate isotype controls to control for non-specific binding !!! Important !!!

Reference: Medina et al, Blood 95:2059, 2000. (Paul Kincade's lab).