Immunofluorescence for DT40

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Centrifuge cells at 1,000 rpm for 5min.

Resuspend cells in 1-10 ml of 1x PBS (10^5-10^6/ml).

Cytospin 300µl of cells at 800 rpm for 5 min and remove the slides immediately for fixation.

Fix cells in 3% paraformaldehyde (PFA) in 250 mM HEPES (pH7.4) or in cold methanol for 15 min at room temperature.

Incubate cells with 0.5% NP40 in PBS for 15 min at room temperature.

Rinse the slides in 0.5% BSA in 1xPBS for 5min at 3 times.

Add 100µl of 1st antibody in 0.5%BSA and incubate for 60min at 37°C.

Wash the slides 3 times with 0.5% BSA in PBS for 5min.

Add 100µl of 2nd antibody in 0.5%BSA and incubate for 45min at 37°C.

Wash the slides 3 times with 0.5% BSA in PBS for 5min.

Wash the slides with PBS.

Mount in DAPI (0.1 µg/ml).

Centromere Staining for DT40

Wash cells with PBS (once).

Resuspend cells in 75 mM KCl hypnotic solution, and incubate at room temperature for 10 min (at a cell density of 2-5x10^5 cells/ml).

Centrifuge cells at 1,000rpm for 5min.

Resuspend cells in cold methanol (10^6-10^7/ml)

Drop 30µl aliquot of this suspension onto a slide glass.

Place slides in a coplin jar containing cold methanol for 30 min. (Timing is very important. If Cells were dried completely, you may get weak or no signals. However, cells should be dried.)

((Acetone wash)) Not necessary.

Flood gently with KCM (120 mM KCl, 20mM NaCl, 10mM Tris-HCl (PH8.0), 0.5mM Na2EDTA, and 0.1% (v/v) Triton X-100) for 15min at room temperature.

Dry slides briefly (Do not dry completely.)

Add 50µl antibody diluted in 1xTEEN (1 mM triethanolamine-HCl pH8.5, 0.2mM Na2EDTA, 25mM NaCl, 0.1% Triton X-100, and 0.1% BSA), and incubate for 60 min at 37°C.

Wash very gently three times by flooding in 1xKB-(10mM Tris-HCl (pH7.7), 0.15M NaCl, and 0.1% BSA) for 3min at room temperature.

Add 50 µl X-conjugated anti rabbit (or human) IgG in 1xKB-(see above), and incubate at 37°C for 45 min.

Wash very gently by flooding in 1xKB-(see above) for 2min at 37 °C.

Fix with 3% paraformaldehyde (PFA) in 250 mM HEPES (pH7.4) for 10 min at room temperature.

Rinse the slides twice with PBS for 3min (if cells are not processed for FISH).

Mount in DAPI (0.1 µg/ml).

Note: If you want to stain metaphase chromosomes with antibodies against centromere proteins, you should treat cells with colcemid. The method described is applicable for staining both mitotic and interphase centromeres.

Metaphase CENP-C Staining for DT40

Wash the cell with PBS (once).

Resuspend the cell in 75 mM KCl hypnotic solution, and incubate at room temperature for 10 min (at a cell density of 2-5x10^5 cells/ml)

Cytospin 200-300 µl of cells at 800 rpm for 10 min at high acceleration, and remove the slides immediately.

Place slides in a coplin jar containing 3:1 methanol/acetic acid (-20°C) for more than 30 min.

((Acetone wash))

Flood gently with KCM (120 mM KCl, 20mM NaCl, 10mM Tris-HCl (PH8.0), 0.5mM Na2EDTA, 0.1% (v/v) Triton X-100) 15min at room temperature.

Dry slides briefly.

Add 50µl antibody diluted in 1xTEEN(1 mM triethanolamine-HCl pH8.5, 0.2mM Na2EDTA, 25mM NaCl, 0.1% Triton X-100, 0.1% BSA), and incubate for 30 min at 37°C.

Wash very gently three times by flooding in 1xKB-(10mM Tris-HCl pH7.7, 0.15M NaCl, 0.1% BSA) for 3 min at room temperature.

Add 50 µl X-conjugated anti rabbit (or human) IgG in 1xKB-(see above), and incubate at 37°C for 30 min.

Wash very gently by flooding in 1xKB-(see above) for 2min at 37 °C.

Fix with 10% formalin in KCM for 10 min at room temperature.

Rinse the slides twice with PBS for 3min (if cells are not processed for FISH).

Mount in DAPI (0.1 µg/ml).