

DNA Staining for Flow Cytometry (HBSS)

Materials:

Staining medium (SM) with azide
80% Ethanol
2 mg/ml RNase solution
0.1 mg/ml PI solution
Nitex mesh (85- μ m pore size)
FACS tubes [Falcon 2052]

PREPARE IN ADVANCE:

1. RNASE solution: 2mg/ml RNase A (Bovine Pancreas Type II, Sigma) in HBSS. Divide into 500ul aliquots and freeze.
2. Propidium iodide solution: 0.1mg/ml PI (Sigma) in HBSS with 0.6% NP-40.
3. HBSS Staining Medium (SM) with 2% calf serum

Protocol:

1. Collect 2×10^6 cells by centrifugation at 400 xg for 5 minutes at 4°C. Aspirate supernatant.
2. Spin and re-suspend pellet in 50 μ l SM/CS and then add it to a conical polypropylene tube containing 1 ml of ice-cold 80% ethanol. Vortex quickly. Fix for 30 minutes to overnight at 4°C.
3. Collect fixed cells by centrifugation at 400 xg for 5 minutes at 4°C. Aspirate supernatant. Re-suspend cell pellet in 500 μ l 2 mg/ml RNase A solution. Incubate for 5 minutes at room temperature.
4. Add 500 μ l 0.1 mg/ml PI solution. Vortex to mix. Incubate for 30 minutes at room temperature, protected from light.
5. Centrifuge at 400 xg for 5 minutes at 4°C. Re-suspend cells in 500 μ l SM by gentle vortexing.
6. Filter cell suspension through Nitex into FACS tubes.
7. Collect data on cytometer using CELLFIT software.

Reagents:

Staining medium (SM) with azide

1X Hank's balanced salt solution (HBSS) [InVitrogen/Gibco 14025-092]
10 mM HEPES•NaOH, pH 7.2
2% (v/v) calf serum
10 mM NaN₃
To 480 ml sterile 1X HBSS add 5 ml sterile 1 M HEPES•NaOH, pH 7.2, 10 ml sterile FBS and 5 ml sterile 1 M NaN₃. Store at 4°C.

2 mg/ml RNase solution

Prepare DNase-free RNase from bovine pancreatic RNase, type IIA [Sigma R 5000]. Dissolve RNase at 10 mg/ml in 10 mM sodium acetate, pH 5.2. Heat at 100°C for 15 minutes. Allow to cool to room temperature. Adjust pH to 7.4 by the addition of $\frac{1}{10}$ volume 1 M Tris-HCl, pH 7.4. Aliquot and store at -20°C.

Alternatively, purchase RNase A solution from Qiagen (100mg/ml stock, 17,500 units, Cat #19101). This stock can be stored at room temperature.

0.1 mg/ml PI solution

0.1 mg/ml propidium iodide [Molecular Probes, P1304]

HBSS (no serum)

0.6% (w/v) NP-40

Aliquot at 500 μ l/aliquot and store, protected from light, at -20°C.