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PLATING MEDIA:

DMEM with 4.5 g/L glucose and 4 mM glutamine (Millipore SLM-020-A)
10% heat-inactivated FBS
1x Nonessential amino acids (from 100x stock, Millipore TMS-001-C)
10mM HEPES (from 1 M HEPES, Millipore TMS-003-C)
100 U/mL Pen-Strep (from 100x stock, Millipore TMS-AB2-C)

PRESENTATION:

Cells are frozen at 1×10^7 cells/mL in DMEM/20% fetal bovine serum/100 U/ml penicillin and streptomycin/10% DMSO.

STORAGE:

Place cells in liquid nitrogen immediately upon receipt. Maintain frozen in liquid nitrogen for up to 5 years.

ASSAY PROTOCOL:

- 1) Thaw cells rapidly by removing from liquid nitrogen and immediately immersing in a 37°C water bath. Immediately after ice has thawed, sterilize the exterior of the vial with 70% ethanol.
- 2) Transfer contents of the vial to a sterile 15 mL conical tube. Add 10 mL prewarmed plating media to the cells and mix gently to resuspend cells. Centrifuge at 200 x g. Remove all but 0.5 mL media.
- 3) Resuspend cells to 0.5×10^6 cells/mL in plating media. Dispense the cell suspension into a 96-well assay plate at 200 μ L per well to obtain a density of approximately 1×10^5 cells/well.
- 4) Place the assay plate in a humidified 37°C incubator with 5% CO₂.
- 5) The cells may be assayed 16-24 hours after plating. It is recommended to wash the cells with assay buffer at least once prior to addition of loading dye.

REFERENCES:

Ali S, Davis MG, Becker MW, Dorn GW (1993) Thromboxane A₂ stimulates vascular smooth muscle hypertrophy by unregulating the synthesis and release of endogenous basic fibroblast growth factor. *J Biol Chem* 268:17397–17403.

Hanasaki K, Nakano K, Kasai H, Arita H (1989) Biochemical characterization and comparison of rat thromboxane A₂/prostaglandin H₂ receptors in platelets and cultures aortic smooth muscle cells. *Biochem Pharmacol* 38:2967–2976.

Hirata M et al. (1991) Cloning and expression of the cDNA for a human thromboxane A₂ receptor gene. *Nature* 349: 617-620.

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