AXOL
Discovery Stems From Here

Transient Transfection of Assay Ready Expanded (ARE) Hepatocytes

Protocol for use

Product Information

Catalog. No.	Product Name	Format	Stock Conc.	Storage on Arrival	Thawing Instructions	Storage Once Thawed
ax3701	Assay-Ready Expanded (ARE) Hepatocytes	5,000,000 cells/vial	N/A	Liquid Nitrogen	Follow protocol	N/A
ax3705	Assay-Ready Expanded (ARE) Hepatocyte Thawing Medium	50mL	N/A	4°C	N/A	N/A
ax3710	Assay-Ready Expanded (ARE) Hepatocyte Maintenance Medium	500mL	N/A	4°C	N/A	N/A
ax3799	Collagen Coated (type I) culture plates	5 x 96 well plates	N/A	4°C	N/A	N/A

Catalog. No.	Product Name
N/A	Plasmid DNA, 1ug/ul in water or TAE buffer
VR-01LB-00	Viromer Red (Lipocalyx)
N/A	Foetal bovine serum
N/A	Phosphate-buffered saline (PBS without Ca2+/Mg2+)
N/A	Trypsin/EDTA (0.05%/0.02%)

Axol ARE Hepatocyte Transfection Using Viromer Red Protocol

Preparation of reagents

Before transfection:

Thaw and expand ARE hepatocytes in ARE Hepatocyte Culture Media as described in product instruction manual. Cells should be cultured for at least 5 days before transfection. Cells will be seeded and transfected on the same day. We recommend adding negative (mock-transfected) and positive (GFP-transfected) controls to your experiment.

Transfection Protocol (single reaction, 6-well format)

Thaw and expand ARE hepatocytes in ARE Hepatocyte Culture Media as described in product instruction manual. Cells should be cultured for at least 5 days before transfection. Cells will be seeded and transfected on the same day. We recommend adding negative (mock-transfected) and positive (GFP-transfected) controls to your experiment.

- In the morning: seed 200,000 cells in 2 ml culture medium per well of a collagen-coated 6-well plate
- Incubate the cells for 4 h at 37°C and 5% CO2 for sufficient adhesion
- Prepare the transfection complexes:
- **Tube 1:** Pipette 178 µl buffer Red and add 2 µl of DNA (1 µg/µl, final DNA conc. 11 ng/µl)
- Vortex for 3 sec
- **Tube 2:** Pipette 0.8 µl Viromer Red and add 19.2 µl buffer Red (critical order, Viromer first)
- Vortex for 3 sec
- Add 180 µl from tube 1 to tube 2 (critical order, always add DNA to reagent)
- Mix swiftly
- Incubate for 15 min at room temperature
- Add 200 µl of transfection complexes dropwise to the cells
- Place the cells on an orbital shaker and shake at 100 rpm for three hours at 37°C and 5% CO2
- Further incubate the cells under static conditions at 37°C and 5% CO2 overnight
- Next day in the morning, replace the transfection medium with fresh ARE Hepatocyte Maintenance Medium
- Verify expression after 2 days, GFP was observed to peak at 48 h post transfection (40-50%)

Notes

Got any questions? Need help with the protocol?
Contact Axol Technical Support at
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Or
call +44 (0) 1223 751051