

## 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%)		



## 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.

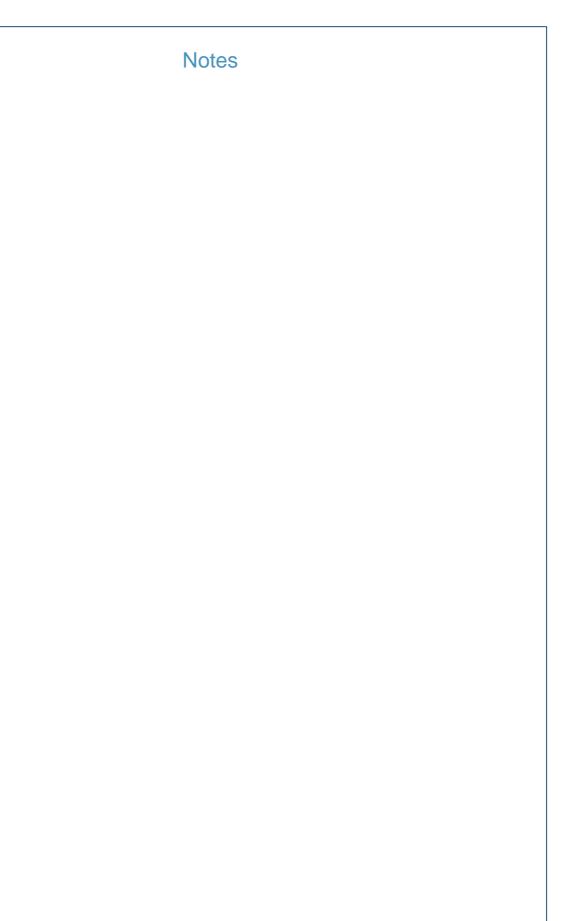
## Transfection Protocol (single reaction, 6-well format)

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%)









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