

Top Tips for ADME-Tox

Techniques and best practice

The following are some key best practices in ADME-Tox in vitro techniques that BioreclamationIVT has gleaned from being a pioneer and market leader in this field since 1990.

Tip 1 Follow the correct protocol

- Different manufacturers = different protocols
- Protocols are optimized for product characteristics

The correct protocol will give better results
Use *InVitroGRO*™ hepatocyte medium

Tip 2 Store and thaw the cells under appropriate conditions

- Store cells with liquid nitrogen at < -150°C in either liquid or vapor phase of liquid nitrogen
- Transport cells under dry ice for less than 30 minutes
- Thaw cells promptly upon removal from the storage unit
- Thaw cells in media pre-warmed to 37°C

Improper storage or thawing temperature degrades cell viability and performance

Tip 3 Minimize assay preparation

- Choose cell lots with high viability by checking the manufacturer's tested viability or by performing the assay yourself
- BioreclamationIVT cells are guaranteed at **5 million viable cells/vial** and **>70% viability** without Percoll® purification
- Cryoplateable Hepatocytes do not require a centrifugation and washing step prior to plating

Fewer steps means less time is required for cryoplateable hepatocytes to run your assays

Tip 4 Do not allow the cells to settle

- Hepatocytes are heavy and settle rapidly
- Invert gently for consistent aliquots

Homogenous aliquots result in consistent cell counts and seeding



0 min.

1 min.

2 min.

3 min.

4 min.

5 min.

Tip 5 Handle hepatocytes gently

- Hepatocytes are fragile
- Avoid rapid pipetting or trituration of the cells

Improper handling can affect cell viability and performance

Tip 6 Measure volumes precisely

- Accuracy is important when filling hemacytometer chambers for cell counting
- Add exactly 10 µL into each chamber

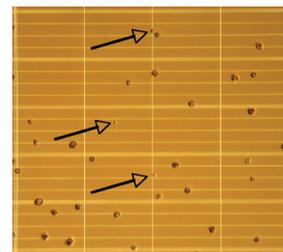
Varying volumes can result in more than 15% error in cell counts and can impact your results

Top Tips for ADME-Tox

Tip 7 Count the correct cells in the appropriate manner

- Additional cell types may be present
- Do not count the smaller non-parenchymal cells
- Only count 2 of the 4 sides within the square

Errors in cell counts can impact your results



Tip 8 Use the correct Trypan Blue concentration

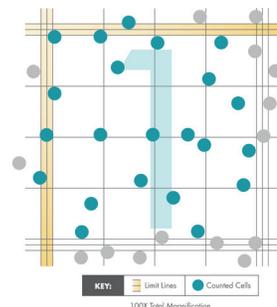
- Use the recommended 1:5 dilution of Trypan Blue

Trypan Blue is toxic to cells at higher concentrations and will affect viability

Tip 9 Count the cells quickly

- After the Trypan Blue is added, count the cells within 1 - 2 minutes

Trypan Blue is toxic to cells at longer exposure times



Tip 10 Distribute the cells evenly

- As a final step to plating, gently shake the plate in a north-south and then east-west direction to distribute the cells evenly

Even distribution of cells results in better attachment

Tip 11 Remove loose cells

- When washing, tap the plate lightly to help suspend the loose cells

Unattached cells may affect plated cells and reduce their adherence



Tip 12 Add media carefully to plated cell

- Monolayers of plated hepatocytes are fragile
- Carefully pipette media down the sides of the wells

Rough handling will disrupt the hepatocyte monolayer and affect experimental outcomes

Tip 13 Avoid pooling issues

- When pooling hepatocytes from multiple lots, work quickly and efficiently
- Avoid delays in thawing, mixing, counting, and plating
- To avoid these problems and reduce waste, consider pre-pooled products like BioreclamationIVT's Liverpool™ Pooled Cryopreserved Human Hepatocytes

Delays in thawing, mixing, counting and plating can affect cell viability



Tip 14 Ask for help when needed

- Call your BioreclamationIVT Scientific Advisors
- Request an in-house product demonstration or seminar

Save time and money by relying on the BioreclamationIVT experts for technical advice on your ADME-Tox assays

Please contact BioreclamationIVT for any questions on ADME-Tox techniques, best practices, applications, products, and services

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