Primary Mouse Hepatocyte Isolation

Two-step collagenase perfusion method via vena cava cannulation

← All Protocols

Overview | Materials | Preparation | Protocol | Troubleshooting

Overview

This protocol describes isolation of primary hepatocytes from mouse liver using a two-step collagenase perfusion technique with retrograde perfusion through the inferior vena cava. Viable hepatocytes are purified using Percoll density gradient centrifugation.

Expected Yield:

- **30-50 × 10^6 viable hepatocytes** per mouse
- **Viability:** Typically >95%
- **Purity:** >90% hepatocytes
- **Duration:** ~2-3 hours per mouse

Applications

- Drug metabolism studies
- Hepatotoxicity assays
- Metabolic pathway analysis
- Primary hepatocyte culture
- Liver disease modeling
- Gene expression studies

Principle

- **Step 1:** Ca^{2+}-free EGTA perfusion disrupts cell-cell junctions
- **Step 2:** Liberase TM digests extracellular matrix

- **Purification:** Percoll gradient separates viable hepatocytes
- **Viability:** Trypan blue exclusion assay

Safety & Animal Care

- Ensure proper IACUC approval for all animal procedures
- Follow institutional guidelines for animal handling and euthanasia
- Wear appropriate PPE: lab coat, gloves, eye protection
- Work in biosafety cabinet for sterile isolation
- Dispose of biological waste properly
- Liberase and enzymes - handle according to SDS