Development and Validation of a Novel Post-Thaw Recovery Media for Human Cryopreserved Hepatocytes

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Abstract

Human hepatocytes provide the most physiologically relevant model for studying hepatic metabolism since they contain the full complement of enzymes and transporter proteins. With progress in cryopreservation, cryopreserved hepatocytes have become an "off-the-shelf" reagent with many advantages including consistency and flexibility. A high post-thaw viability is required for successful studies, so it is critical to have both an effective cryohepatocyte recovery media and high-quality cryopreserved hepatocytes.

BD Biosciences has developed and validated a new post-thaw recovery medium for human cryopreserved hepatocytes. The recovery medium maintains hepatocyte post-thaw functions while improving cell health and recovery. The newly developed recovery medium was optimized with BD Gentest™ human inducible cryohepatocytes for viability and recovery. It showed an average 5% post-thaw viability increase compared to traditional Percoll™ methods with more than 20 lots tested. Cell recovery increased on average 1.5 million cells/vial compared to Percoll (24% increase). Twenty-four hour cell confluency and morphology were slightly better with the new recovery media while 8-hour midazolam metabolism and CYP3A4 basal and fold-induction remained consistent. One tube of the recovery medium was able to recover up to 5 vials of cryopreserved hepatocytes. The results indicate that the previous method of recovering cryopreserved hepatocytes, like Percoll recovery method, was not optimal. The newly developed post-thaw recovery medium is shown to consistently provide improved cell health and recovery while maintaining hepatocyte function. The new recovery medium has other advantages such as requiring only one centrifugation and the ability to recover multiple vials without sacrifice in viability and recovery.

Introduction

Primary human hepatocytes contain the full complement of hepatic drug metabolism enzymes and transporter proteins, and is the most physiological relevant in vitro system for predictive IVIVE and comprehensive identification of metabolites in preclinical species and human. Although fresh plated hepatocytes are still widely used, cryopreserved human hepatocytes that plate and induce for CYPs are becoming more popular, as they have the advantage of end-user convenience and availability of multiple lots. The plating efficiency and cell morphology of plated cryopreserved hepatocytes is dependent to a large extent on the post-thaw recovery procedure, which includes the choice of both the post-thaw recovery medium and cell plating medium. In order to maximize post-thaw cell viability, recovery, and plating efficiency, BD Biosciences has developed a new proprietary non-cytotoxic (non-Percoll) recovery medium. Percoll has been shown to be harmful to certain cell types due to contamination by endotoxins and polyvinylpyrrolidine (the chemical agent for stabilizing the colloidal-silica Percoll core). Consequently, Percoll has been withdrawn from the market for clinical applications. With the newly developed recovery medium, cells can be directly plated after a single centrifugation step versus a two-step centrifugation method which is routinely used with Percoll-based recovery mediums. The comparison between the new recovery medium and Percoll medium showed the new recovery medium consistently improved post-thaw viability, recovery, and plating efficiency, while CYP3A4 induction and midazolam clearance results were similar between the two mediums. Up to five vials of cryopreserved hepatocytes can be processed using a single tube of recovery media without loss in cell viability or recovery, which saves researchers time when conducting large-scale experiments.

Materials and Methods

Materials

BD Gentest™ high viability cryohepatocyte recovery kit (cat. no. 454534): the kit contains 2 tubes (45 ml each) of the new recovery medium and 2 tubes (45 ml each) of plating medium. Percoll recovery media was 30% isotonic Percoll in cell culture media. Human hepatocytes were BD Gentest induction-qualified cryopreserved hepatocytes (cat. nos. 454550 and 454551). The thawing and plating procedures were per the instructions provided with the products.

CYP3A4 Induction Assay

Cells were diluted 1.0x10^6 cells/mL and seeded on 24-well plates (BD BioCoat™ collagen I plates–cat. no. 354408) at a density of 400,000 cells/well. Plate was incubated at 37ºC with 5% CO_2 for 4 hours before the media was changed to supplemented BD™ hepatocyte culture media. The plate was kept in the incubator overnight. Induction was conducted by adding 400 µL/well supplemented hepatocyte culture media containing 20 µM rifampicin daily from day 2 to day 4. Same amount of DMSO was added as vehicle control to obtain basal activities. On day 5, 200 µM testosterone was added and incubated with hepatocytes for 30 minutes. Supernatants were then collected into tubes containing stop solution and protein samples were collected by incubating cells with 1% SDS solution for 15 minutes. Metabolite samples were analyzed by HPLC and protein concentration was performed by Lowry assay. The enzyme activity was expressed as pmol/mg protein/min. Each data point represents mean of 3 wells.

Midazolam Assay

Cells were seeded on a 48-well plate at a seeding density of 0.168x10^6 cells/well, and were incubated at 37ºC/5% CO_2 incubator. Four hours later, the seeding medium was aspirated and an incubation medium added. After an overnight incubation, the incubation medium was aspirated and 100 µl medium containing 5 µM Midazolam was added. Incubation was terminated at different time points (0, 0.5, 1, 2, 3, 4, 6, and 8 hours). Cells were scraped from plates and kept at -20ºC for later HPLC or LC/MS analysis.

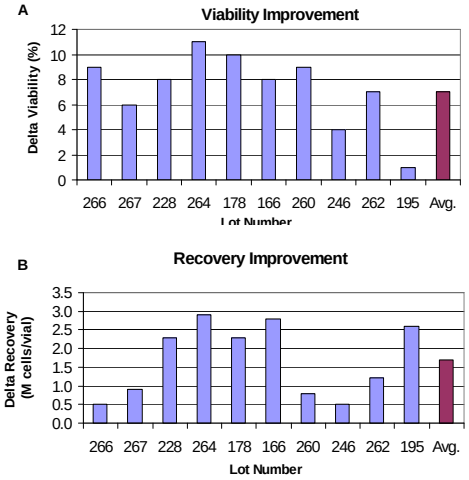
Summary

The BD Gentest high viability cryohepatocyte recovery kit (cat. no. 454534)

- Improves cryopreserved hepatocyte cell viability, yield, and plating efficiency
- Saves researchers time with an optimized single centrifugation recovery step versus traditional Percoll two-step processes
- Recovers up to 5 vials of cryopreserved hepatocytes per purification
- Supports human platable cryopreserved hepatocyte recovery and plating for induction assays or other assays requiring plated cells such as metabolism, transporter, or toxicity studies

Results

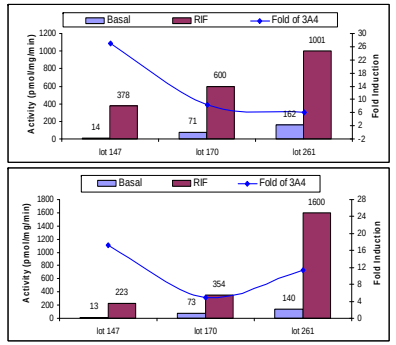
Viability and Recovery Improvement Comparing to Percoll Recovery Media



(**A**) Post-thaw viability increased 7% on average for a representative 10 BD Gentest inducible cryopreserved hepatocyte lots when the BD Gentest high viability cryohepatocyte recovery media was compared to conventional Percoll recovery media. (**B**) Post-thaw recovery increased 1.7 million cells/vial on average (25% increase) for the same 10 lots.

Induction Assay and Midazolam Assay

A

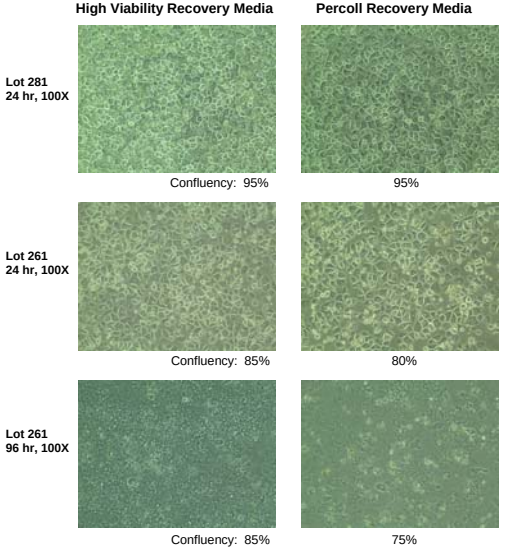


B

Recovery Media	Seeding density (viable cells/ml)	Midazolam clearance (ml/hr/e6 cell)	%MDZ remaining @8hr
High Viability Recovery Media	1.00E+06	0.204	5.1
Percoll Recovery Media	1.00E+06	0.201	9.3

BD Inducible hepatocytes were thawed and purified side-by-side with the new high viability recovery media and Percoll recovery media. 3A4 induction assays (**A**) and Midazolam metabolism assays (**B**) indicated the new high viability recovery media and Percoll recovery media gave similar assay results with the same lot of hepatocytes.

Side-By-Side Plating Efficiency



The BD Gentest high viability cryohepatocyte recovery media maximizes plating efficiency when compared to Percoll recovery media. Confluency was determined by visual inspection.

Maximum Number of Vials per Purification

No. vials of cryopreserved hepatocytes used per tube of high viability recovery media	Lot 251 Viability (%)	Lot 251 Recovery (M/vial)	Lot 247 Viability (%)	Lot 247 Recovery (M/vial)
1	84.2	6.7	80.8	11.8
2	84.5	7.4	79	12.3
3	83.9	6.8	81.7	12.5
4	81.3	6.6	76.7	12.3
5	81.3	6.8	80.3	13.3
6	78.4	6.5	75.5	12.4

Post-thaw viability and recovery using up to 5 vials of cryohepatocytes per tube of high viability recovery media demonstrates the equivalent viability and recovery as individually thawed tubes of cryohepatocytes. Greater than 5 vials (6) of cryopreserved hepatocytes per tube of recovery media demonstrated a 5% drop in viability.

Benchmark

	Lot 142 Viability (%)	Lot 142 Recovery (M/vial)	Lot 170 Viability (%)	Lot 170 Recovery (M/vial)	Lot 261 Viability (%)	Lot 261 Recovery (M/vial)
High Viability Recovery Kit	95	7.3	84	2.9	73	4.3
BD Percoll Kit	90	3.7	81	3.1	50	2.7
Competitor 1	91	7.5	74	4.4	58	5.5
Competitor 2	64	9.1	49	7.9	30	5.4

Primary criteria is viability, as it is an indicative of cell health and maximized plating efficiency.

Lot-to-Lot Consistency

	Viability (%) Kit Lot 1	Viability (%) Kit Lot 2	Viability (%) Kit Lot 3	Recovery (M/vial) Kit Lot 1	Recovery (M/vial) Kit Lot 2	Recovery (M/vial) Kit Lot 3
Lot 178	89.0	88.6	85.4	6.89	6.71	8.44
Lot 246	83.9	85.7	84.9	10.85	11.45	10.65
Lot 260	88.8	89.7	89.1	6.42	5.33	6.73

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