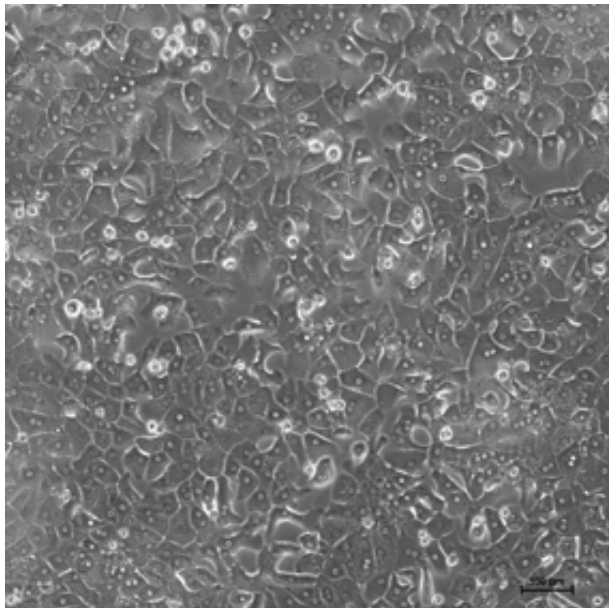
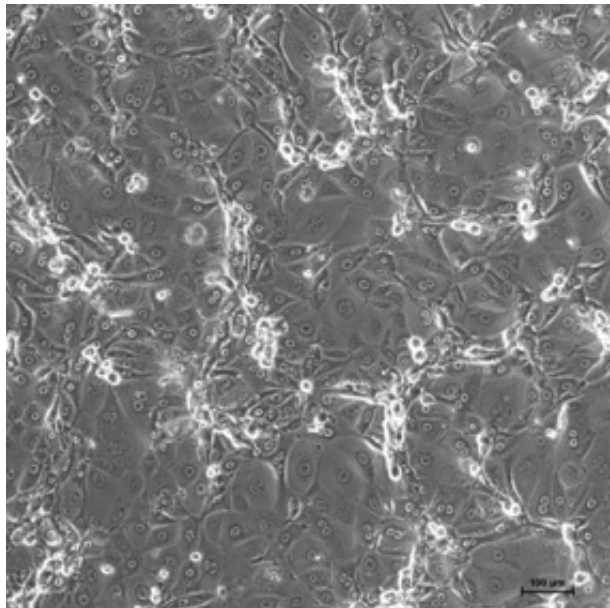


| BHCP-I Cryopreserved Plateable Beagle Hepatocytes for Induction assays | | | | | | | | |
|---|------------------|--|-----------------|------------------|---------------------|------|----------------------|------|
| Cell Specification | | | | | | | | |
| Lot BH180829 | | Batch Release: November 16, 2018 – Updated: Dec 16, 2020 | | | | | | |
| Species: Beagle | | Gender: male Age: 7.5 months | | | | | | |
| Cryopreservation: Date: August 29, 2018 Amount per vial: 10.5 x 10 ⁶ cells | | Thawing: n=3 Post-thaw viability: 90.4 ± 2.3 % Post-thaw yield per vial: 7.1 ± 1.8 x 10 ⁶ cells Recovery: 67 ± 18 % | | | | | | |
| Phase contrast on day 1 after thawing (24well plate) | | Phase contrast on day 3 after thawing (24well plate) | | | | | | |
|  | |  | | | | | | |
| Recommended seeding density on collagen-coated plates: 12well plate – 800,000 cells/well 24well plate – 400,000 cells/well 96well plate – 90,000 cells/well Culture in Human Hepatocyte Maintenance Medium (HHMM). | | | | | | | | |
| CYP P450 activity in culture after thawing: Ethoxyresorufin-O-deethylation: Induction with 25 µM beta-naphthoflavone | | <table><tr><td>pmol/(mg × min)</td><td>X-fold induction</td></tr><tr><td>24well: 107.9 ± 5.8</td><td>13.2</td></tr><tr><td>96well: 198.9 ± 16.7</td><td>18.0</td></tr></table> | pmol/(mg × min) | X-fold induction | 24well: 107.9 ± 5.8 | 13.2 | 96well: 198.9 ± 16.7 | 18.0 |
| pmol/(mg × min) | X-fold induction | | | | | | | |
| 24well: 107.9 ± 5.8 | 13.2 | | | | | | | |
| 96well: 198.9 ± 16.7 | 18.0 | | | | | | | |

Determination of CYP activities in suspension (Eppendorf Thermomixer C, 1000 rpm at 37 °C with 0.5 x 10⁶ cells in 0.5 ml HPM Cryo medium with 5 % FCS):

| Assay | Enzyme activities (pmol/min*mg protein) mean ± SD |
|-----------------------------|---|
| Phenacetin-O-deethylase | 70.3 ± 1.4 |
| Bupropion-hydroxylase | 21.7 ± 0.6 |
| Midazolam 1'-hydroxylase | 47.8 ± 3.8 |
| UDP-Glucuronosyltransferase | 180.1 ± 3.8 |
| Sulfotransferase | 44.5 ± 1.2 |

Note: Yield, viability, recovery and activity assays were performed at PRIMACYT using PRIMACYT's manual for thawing, plating and culture of primary cryopreserved hepatocytes.

Store at -150 °C or in the vapour phase of LN₂.

This product should be considered as potential biohazard. Only intended for *in vitro* use.

Issued by M. Thiede
Updated by A. Ullrich

Verified by C. Garve