

RTHCP-I Rainbow Trout Hepatocytes Cryopreserved Plateable and CYP Inducible Cell Specification – Certificate of Analysis (CoA)

Lot RTH230613

Batch Release: March 18, 2026

Donor data

Species: Rainbow trout (*Oncorhynchus mykiss*)

Gender: female
Age: sexual immature
Pool: n = 6

Animal characteristics

Donor	1	2	3	4	5	6
Fish weight (g)	297	365	337	357	319	415
Fish length (cm)	28	30	32	31	29	34
Gonads weight (g)	0.25	0.31	0.28	0.37	0.26	1.24
GSI (gonads weight/fish weight)	0.08	0.09	0.08	0.10	0.08	0.30
Liver weight (g)	4.9	4.9	3.0	3.6	3.2	6.9
Total liver weight (g)	26.5					

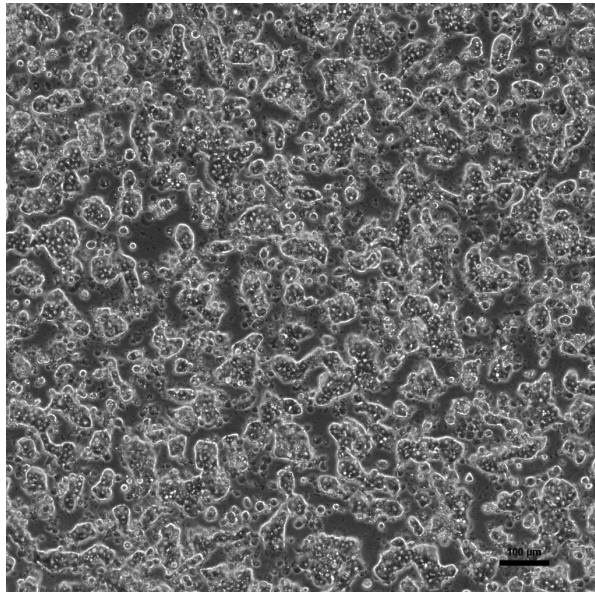
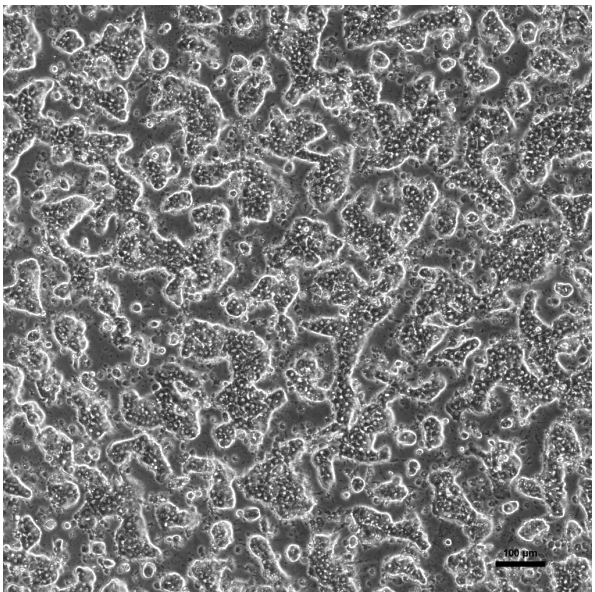
Cryopreservation and Thawing

Cryopreservation:

Date: June 13, 2023
Amount per vial: 15.0 x 10⁶ cells

Thawing: n=5

Post-thaw viability: 96.0 ± 1.5 %
Post-thaw yield per vial: 3.6 ± 0.8 x 10⁶ cells
Recovery: 24 %

2D culture										
Phase contrast on day 6 after thawing (24well plate)	Phase contrast on day 10 after thawing (24well plate)									
										
<p>Recommended seeding density on Corning Primaria plates coated with Matrigel: 24well plate – 500,000 cells/well Culture in Fish Hepatocytes Medium (FHM).</p>										
CYP P450 activity in 2D culture after thawing: Ethoxyresorufin-O-deethylation: Induction with 18 µM β-Naphthoflavone	pmol/(mg × min) 24well: 8.7 ± 0.3	X-fold induction 2.7								
<p>Phase I metabolism: Determination of basal enzymatic activities in plated cells:</p> <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="text-align: center;">Assay</th> <th style="text-align: center;">Enzyme activities (nM/min*mg protein) mean ± SD</th> </tr> </thead> <tbody> <tr> <td style="text-align: center;">Phenacetin-O-deethylase</td> <td style="text-align: center;">0.51 ± 0.03</td> </tr> <tr> <td style="text-align: center;">Diclofenac-hydroxylase</td> <td style="text-align: center;">0.78 ± 0.04</td> </tr> <tr> <td style="text-align: center;">Midazolam 1'-hydroxylase</td> <td style="text-align: center;">0.12 ± 0.001</td> </tr> </tbody> </table> <p>Enzyme activity assays were performed at PRIMACYT GmbH. The assays were conducted with 0.5 × 10⁶ plated cells in 0.5 mL FHM at 15 °C for 2 h. Values for enzyme activities are mean ± standard deviation of two determinations. Metabolite formation was determined with validated LC-MS/MS methods by a GLP certified external service provider.</p>			Assay	Enzyme activities (nM/min*mg protein) mean ± SD	Phenacetin-O-deethylase	0.51 ± 0.03	Diclofenac-hydroxylase	0.78 ± 0.04	Midazolam 1'-hydroxylase	0.12 ± 0.001
Assay	Enzyme activities (nM/min*mg protein) mean ± SD									
Phenacetin-O-deethylase	0.51 ± 0.03									
Diclofenac-hydroxylase	0.78 ± 0.04									
Midazolam 1'-hydroxylase	0.12 ± 0.001									

Suspension culture

Viability test on orbital shaker (Eppendorf Thermomixer C, 1000 rpm at 14 °C with 0.5×10^6 cells in 0.5 ml L15-Cryo):

Time (h)	0	1	2	3	4	5	24
Viability (%)	94.6	97.8	99.5	99.4	99.5	99.3	96.3

Phase I and Phase II metabolism: Determination of enzymatic activities in suspension

Assay	Enzyme activities (pmol/min*mg protein) mean \pm SD
Phenacetin-O-deethylase	1.44 \pm 0.27
Bupropion-hydroxylase	0.78 \pm 0.13
Midazolam 1'-hydroxylase	1.54 \pm 0.26
UDP-Glucuronosyltransferase	8.91 \pm 1.15
Sulfotransferase	5.68 \pm 0.25

Enzyme activity assays were performed at PRIMACYT GmbH. The assays were conducted with 0.5×10^6 cells in 0.5 mL L-15 medium with 5 % FCS at 14 °C and 1.000 rpm using an Eppendorf Thermomixer C. Values for enzyme activities were determined at a single substrate concentration and are mean \pm standard deviation of three determinations. Metabolite formation was determined with validated LC-MS/MS methods by a GLP certified external service provider.

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

Detailed animal information and husbandry conditions

Species	Rainbow trout (<i>Oncorhynchus mykiss</i>)
Vendor	Fish Aquaristikshop, Osterberg 11, 19061 Schwerin
Food	Alkote, Allco Heimtierbedarf GmbH & Co. KG, Thedinghausen
Light/Dark cycle	natural day / night cycle using daylight
Husbandry	3.5 m ³ water tank
Stocking rate (kg/m ³)	2.7 \pm 0.3
Water temperature (°C)	15.9 \pm 0.3
pH	8.2 \pm 0.3
NH ₄ (mg/l)	0.2 \pm 0.1
NO ₂ (mg/l)	0.3 \pm 0.2
NO ₃ (mg/l)	5.9 \pm 2.6
Carbonate hardness (°dh)	8.4 \pm 0.6
Salinity (‰)	0.20 \pm 0.00
Conductivity (µS/cm)	568.4 \pm 12.2
Acid capacity pH 4.3 (mmol/l)	3.0 \pm 0.2

Animals were housed under veterinary control and allowed to acclimate ≥ 7 days before use. Liver tissues were obtained from non-infectious, non-contagious, healthy animals. The animals do not originate from a facility conducting work or research with animal pathogens.

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. Reu

Verified by: J. Schuldt