

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

Lot RTH260323

Batch Release: July 22, 2026

Donor data

Species: Rainbow trout (*Oncorhynchus mykiss*)

Gender: female
Age: sexual immature
Pool of 5

Animal characteristics

Donor	1	2	3	4	5
Fish weight (g)	454	323	345	481	373
Fish length (cm)	34	31	34	35	32
Gonads weight (g)	0.7	0.7	0.6	0.7	0.6
GSI (gonads weight/fish weight)	0.15	0.22	0.17	0.15	0.16
Liver weight (g)	5.8	6.5	5.6	4.6	5.3
Total liver weight (g)	27.8				

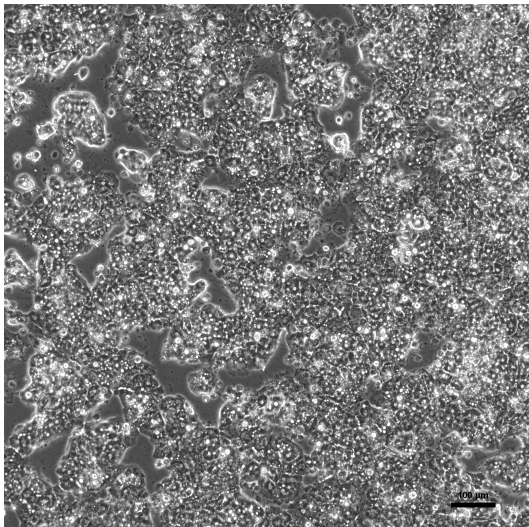
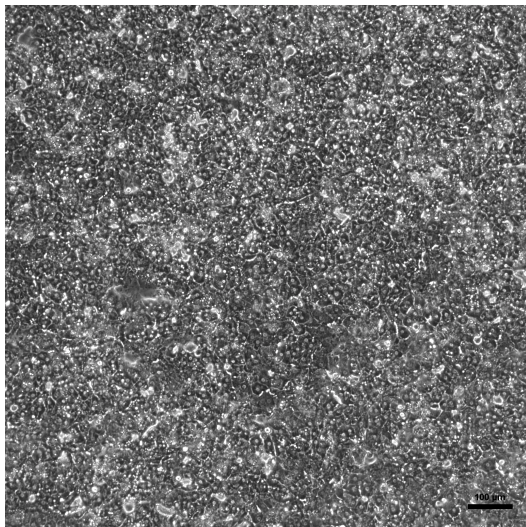
Cryopreservation and Thawing

Cryopreservation:

Date: March 23, 2026
Amount per vial: 18.6×10^6 cells

Thawing: n=3

Post-thaw viability: 99.4 ± 0.3 %
Post-thaw yield per vial: $10.9 \pm 3.1 \times 10^6$ cells
Recovery: 58 %

2D culture		
Phase contrast on day 6 after thawing (24well plate)	Phase contrast on day 9 after thawing (24well plate)	
		
Recommended seeding density on Corning Primaria plates coated with Matrigel: 24well plate – 600,000 cells/well Culture in Fish Hepatocytes Medium (FHM).		
CYP P450 activity in 2D culture after thawing: Ethoxyresorufin-O-deethylation: Induction with 5 µM β-Naphthoflavone for 72 h	pmol/(mg × min) 24well: 2377 ± 30	X-fold induction 42.8
Phase I and Phase II metabolism: Determination of enzymatic activities in plated cells:		
Assay	Enzyme activities (nM/min*mg protein) mean ± SD	
	basal	induced
Phenacetin-O-deethylase	1.54 ± 0.1	78.49 ± 1.64
Diclofenac-hydroxylase	1.99 ± 0.17	1.36 ± 0.02
Midazolam 1'-hydroxylase	1.28 ± 0.0	1.71 ± 0.18
UDP-Glucuronosyltransferase	14.83 ± 0.59	-
Sulfotransferase	4.47 ± 0.26	-

Enzyme activity assays were performed at PRIMACYT GmbH. For phase I metabolism, cells were induced for 72 h with 10 µM β-Naphthoflavone and 10 µM Rifampicin. The assays were conducted with 0.5 x 10⁶ plated cells in 0.5 mL FHM at 15 °C for 1 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.

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 (%)	99.2	98.0	99.2	99.1	99.4	100.0	99.2

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	2.8 ± 0.4
Bupropion-hydroxylase	4.0 ± 0.5
Midazolam 1'-hydroxylase	5.1 ± 0.3
UDP-Glucuronosyltransferase	17.9 ± 1.5
Sulfotransferase	4.5 ± 0.1

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.

Pyrene depletion: Data will follow (including depletion rate constant and in vitro intrinsic clearance)

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	Primacyt GmbH, Hagenower Str. 73, 19061 Schwerin
Food	Alkote, Allco Heimtierbedarf GmbH & Co. KG, Thedinghausen
Light/Dark cycle	natural day / night cycle using daylight
Husbandry	0.7 m ³ water tank
Stocking rate (kg/m ³)	5.8 ± 2.5
Water temperature (°C)	13.9 ± 1.4
pH	7.2 ± 0.3
NH ₄ (mg/l)	0.8 ± 0.7
NO ₂ (mg/l)	2.8 ± 2.4
NO ₃ (mg/l)	5.1 ± 3.9
Carbonate hardness (°dh)	11.9 ± 1.3

Salinity (‰)	0.4 ± 0.3
Conductivity (µS/cm)	984.9 ± 526.7
Acid capacity pH 4.3 (mmol/l)	4.2 ± 0.5
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/A. Ullrich	Verified by: J. Schuldt
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