

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

Lot RTH260324

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)	451	385	305	455	318
Fish length (cm)	35	32	31	35	31
Gonads weight (g)	0.5	0.6	0.5	0.9	0.6
GSI (gonads weight/fish weight)	0.12	0.16	0.16	0.19	0.20
Liver weight (g)	5.6	8.0	4.6	7.8	5.7
Total liver weight (g)	31.7				

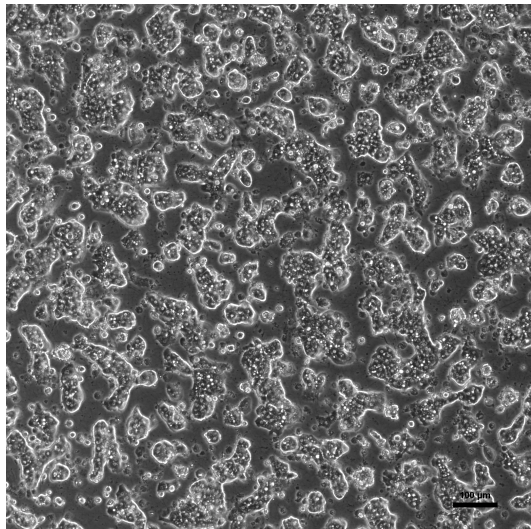
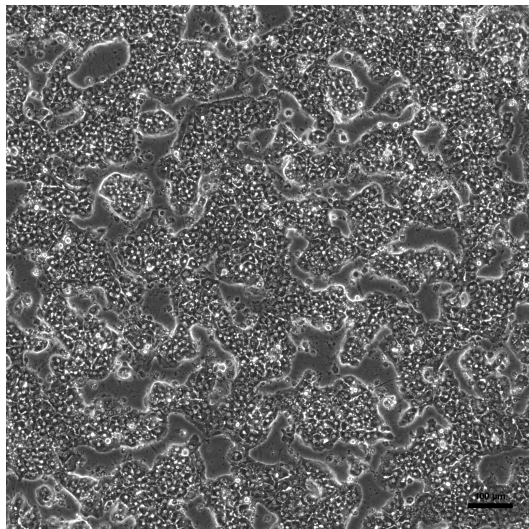
Cryopreservation and Thawing

Cryopreservation:

Date: March 23, 2026
Amount per vial: 15.4×10^6 cells

Thawing: n=4

Post-thaw viability: 98.5 ± 1.3 %
Post-thaw yield per vial: $10.0 \pm 2.6 \times 10^6$ cells
Recovery: 65 %

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	pmol/(mg × min) 24well: 2232 ± 35	X-fold induction 33.4
Phase I metabolism: Determination of basal and induced enzymatic activities in plated cells:		
Assay	Enzyme activities (nM/min*mg protein)	
	mean ± SD	
	basal	induced
Phenacetin-O-deethylase	1.62 ± 0.27	65.85 ± 2.73
Diclofenac-hydroxylase	1.48 ± 0.02	0.87 ± 0.05
Midazolam 1'-hydroxylase	0.99 ± 0.01	1.45 ± 0.01
UDP-Glucuronosyltransferase	17.47 ± 3.5	-
Sulfotransferase	4.67 ± 0.91	-

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 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.

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 (%)	96.8	97.6	99.2	100	99.1	98.7	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	1.9 $\pm$ 0.2
Bupropion-hydroxylase	3.3 $\pm$ 1.0
Midazolam 1'-hydroxylase	4.3 $\pm$ 0.6
UDP-Glucuronosyltransferase	15.0 $\pm$ 2.8
Sulfotransferase	4.3 $\pm$ 0.7

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)	4.3 $\pm$ 3.3
Water temperature (°C)	13.9 $\pm$ 1.3
pH	7.3 $\pm$ 0.3
NH ₄ (mg/l)	0.7 $\pm$ 0.7
NO ₂ (mg/l)	2.8 $\pm$ 2.3
NO ₃ (mg/l)	6.2 $\pm$ 4.9
Carbonate hardness (°dh)	11.9 $\pm$ 1.3

Salinity (‰)	0.4 ± 0.3
Conductivity (µS/cm)	1036.8 ± 517.8
Acid capacity pH 4.3 (mmol/l)	4.2 ± 0.4
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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