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Cell Culture Technology

RTHCP-I Rainbow Trout Hepatocytes Cryopreserved Plateable and CYP Inducible Cell Specification – Certificate of Analysis (CoA)	
Lot RTH260319	Batch Release: July 21, 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)	367	386	432	414	364
Fish length (cm)	33	31	34	33	32
Gonads weight (g)	0.8	0.9	0.7	0.8	0.4
GSI (gonads weight/fish weight)	0.22	0.23	0.16	0.19	0.11
Liver weight (g)	5.9	4.1	6.1	5.7	5.3
Total liver weight (g)	27.1				

Cryopreservation and Thawing	
Cryopreservation: Date: March 19, 2026 Amount per vial: 16.1 x 10^6 cells	**Thawing:** n=3 Post-thaw viability: 98.9 ± 0.2 % Post-thaw yield per vial: 8.9 ± 1.5 x 10^6 cells Recovery: 55 %

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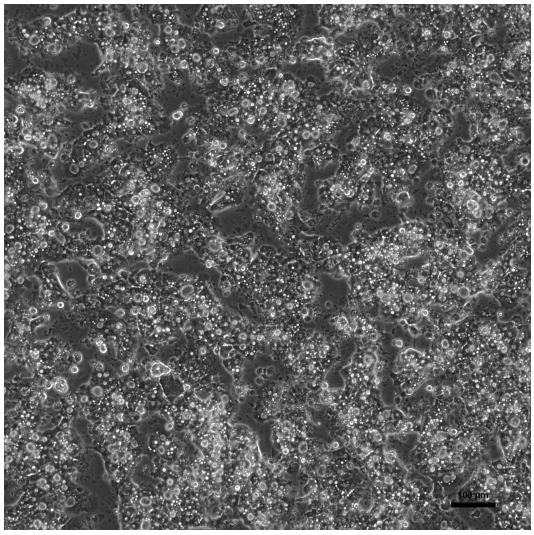
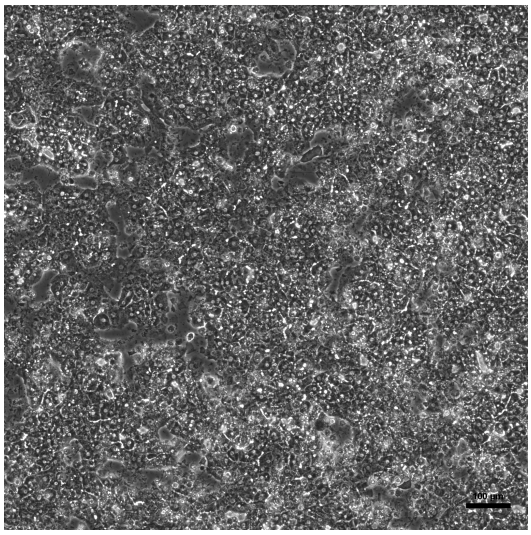
2D culture	
Phase contrast on day 6 after thawing (24well plate)	Phase contrast on day 9 after thawing (24well plate)
	

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 ß-Naphthoflavone for 72 h	pmol/(mg × min) 24well: 1584 ± 75	X-fold induction 25.6

Phase I metabolism: Determination of basal enzymatic activities in plated cells:

Assay	**Enzyme activities (nM/min*mg protein)** mean ± SD
Phenacetin-O-deethylase	2.13 ± 0.24
Diclofenac-hydroxylase	Not detectable
Midazolam 1'-hydroxylase	0.30 ± 0.01
UDP-Glucuronosyltransferase	12.52 ± 1.02
Sulfotransferase	2.27 ± 0.11

Enzyme activity assays were performed at PRIMACYT GmbH. The assays were conducted with 0.5 x 10^6 plated cells in 0.5 mL FHM at 15 °C for 1 h. Values for enzyme activities are mean ± standard deviation of three determinations. Metabolite formation was determined with validated LC-MS/MS methods by a GLP certified external service provider.

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Suspension culture

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

Time (h)	0	1	2	3	4	5	24
Viability (%)	98.7	99.2	97.0	99.4	100.0	98.8	100.0

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

Assay	**Enzyme activities (pmol/min*mg protein)** mean ± SD
Phenacetin-O-deethylase	2.27 ± 0.17
Bupropion-hydroxylase	5.08 ± 0.62
Midazolam 1'-hydroxylase	5.75 ± 0.10
UDP-Glucuronosyltransferase	21.6 ± 1.06
Sulfotransferase	4.90 ± 0.28

Enzyme activity assays were performed at PRIMACYT GmbH. The assays were conducted with 0.5 x 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 ± 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 (Oncorhynchus mykiss)
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)	7.2 ± 1.6
Water temperature (°C)	13.7 ± 1.6
pH	7.2 ± 0.2
NH_4 (mg/l)	0.8 ± 0.7
NO_2 (mg/l)	2.0 ± 2.3
NO_3 (mg/l)	3.5 ± 0.9
Carbonate hardness (°dh)	11.6 ± 1.4

Salinity (‰)	0.3 ± 0.3
Conductivity (µS/cm)	831.2 ± 472.4
Acid capacity pH 4.3 (mmol/l)	4.1 ± 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 LN_2

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

Issued by: M. Reu/A. Ullrich	Verified by: J. Schuldt