

**RTHCP Rainbow Trout Hepatocytes Cryopreserved Plateable
Cell Specification – Certificate of Analysis (CoA)**

Lot RTH230629-1

Batch Release: March 18, 2026

Donor data

Species: Rainbow trout (*Oncorhynchus mykiss*)

Gender: mixed, 3 female, 1 male

Age: sexual immature

Pool: n = 4

Animal characteristics

Donor	1	2	3	4
Fish weight (g)	392	357	378	332
Fish length (cm)	30	31	32	32
Gonads weight (g)	1.22	0.14	0.38	0.40
GSI (gonads weight/fish weight)	0.31	0.04	0.10	0.12
Liver weight (g)	5.2	3.7	3.9	3.2
Total liver weight (g)	16.1			

Cryopreservation and Thawing

Cryopreservation:

Date: June 29, 2023

Amount per vial: 15.0 x 10⁶ cells

Thawing: n=4

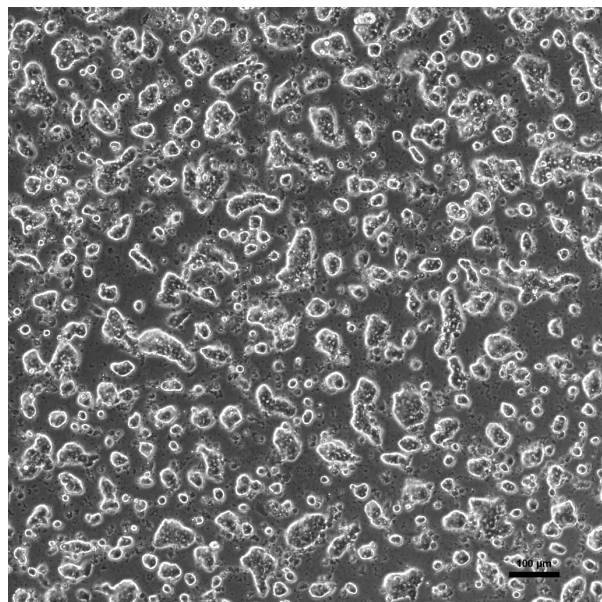
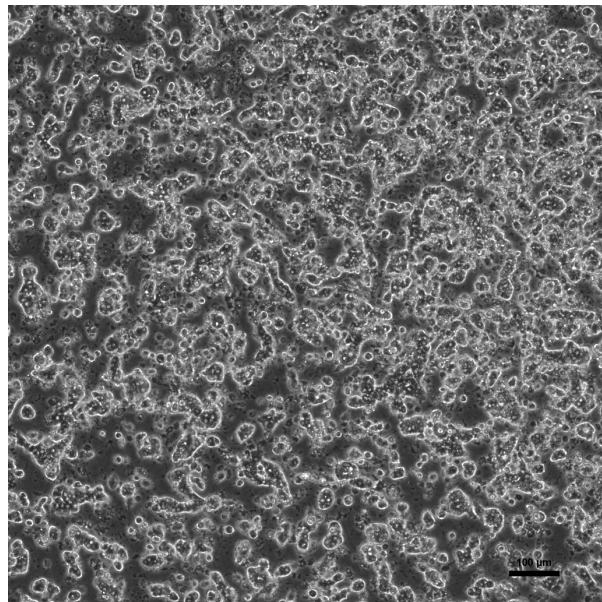
Post-thaw viability: 97.0 ± 1.1 %

Post-thaw yield per vial: 6.0 ± 0.9 x 10⁶ cells

Recovery: 40 %

2D culture

Phase contrast on day 6 after thawing (24well plate)	Phase contrast on day 10 after thawing (24well plate)
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Recommended seeding density on Corning Primaria plates coated with Matrigel:
24well plate – 460,000 cells/well
Culture in Fish Hepatocytes Medium (FHM).

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

Assay	Enzyme activities (nM/min*mg protein) mean ± SD
Phenacetin-O-deethylase	0.47 ± 0.05
Diclofenac-hydroxylase	0.52 ± 0.00
Midazolam 1'-hydroxylase	0.04 ± 0.001

Enzyme activity assays were performed at PRIMACYT GmbH. The assays were conducted with 0.46×10^6 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 (%)	96.4	96.7	98.8	98.3	98.1	99.0	96.1

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	3.37 \pm 0.55
Bupropion-hydroxylase	0.68 \pm 0.15
Midazolam 1'-hydroxylase	1.24 \pm 0.08
UDP-Glucuronosyltransferase	11.85 \pm 0.76
Sulfotransferase	7.63 \pm 0.26

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)	2.2 \pm 0.5
Water temperature (°C)	16.1 \pm 0.5
pH	8.1 \pm 0.4
NH ₄ (mg/l)	0.2 \pm 0.1
NO ₂ (mg/l)	0.2 \pm 0.1
NO ₃ (mg/l)	8.1 \pm 2.4
Carbonate hardness (°dh)	8.4 \pm 0.7
Salinity (‰)	0.2 \pm 0.0
Conductivity (µS/cm)	583.6 \pm 10.8
Acid capacity pH 4.3 (mmol/l)	3.0 \pm 0.3

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