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Cell Culture Technology

RTHCS Cryopreserved Rainbow Trout Hepatocytes for Suspension Assays
Cell Specification

Lot RTH180212-1 Pool | Batch Release: June 1, 2018 – Last Updated Dec 14, 2022

Species: Rainbow trout (Oncorhynchus mykiss)
Strain: Christophersen, Bornhoeved
Supplier: Fish breeding Christophersen
Acclimation temperature: 13.2 ± 1.3 °C
Age: approx. 2 years

Number and gender of animals: 1 female, 2 male sexual immature

All animals were kept under controlled environmental conditions at Fraunhofer EMB in Lübeck.

Animal characteristics:

Donor	1	2	3
Fish weight (g)	360	352	330
Liver weight (g)	6.33	5.35	3.68
Gonad weight (g)	-	0.14	0.46
GSI (gonad weight/fish weight)	-	0.04	0.14

GSI = Gonadosomatic index

Cryopreservation:
Date: February 12, 2018
Amount per vial: 15.0 x 10^6 cells

Thawing: n = 3
Post-thaw viability: 85.6 ± 2.7 %
Post-thaw yield per vial: 2.5 ± 0.8 x 10^6 cells
Recovery: 16.4 %

Viability test: Orbital shaker (Eppendorf Thermomixer C, 1000 rpm at 14 °C with 0.5 x 10^6 cells in 0.5 ml L-15 medium with 5 % FCS):
n = 3

Time (h)	0	0.5	1	1.5	2	3	4	5	15	24
Viability (%)	89.4	89.4	92.7	88.0	96.4	95.7	89.7	96.2	98.7	99.1

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

Assay	Enzyme activities (pmol/min*mg protein) mean ± SD
Phenacetin-O-deethylase	2.6 ± 0.9
Bupropion-hydroxylase	2.2 ± 0.1
Diclofenac 4'-hydroxylase	6.2 ± 1.6
Bufuralol 1'-hydroxylase	1.2 ± 0.1
Midazolam 1'-hydroxylase	4.5 ± 0.3
UDP-Glucuronosyltransferase	32.1 ± 3.4
Sulfotransferase	13.1 ± 1.3

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.

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Determination of Polycyclic Aromatic Hydrocarbons (PAH) metabolism:
Incubation of trout hepatocytes with 20 µM Benzo[a]pyrene for 24 h. Determination of Benzo[a]pyrene metabolites by HPLC-FLD in cell culture medium after treatment with ß-Glucuronidase/Arylsulfatase. Chemical analysis of Benzo[a]pyrene metabolites was performed by Biochemisches Institut für Umweltcarcinogene, Prof. Dr. Gernot Grimmer Stiftung, Großhansdorf, Germany.

Metabolite	**ng/ml**
trans-7,8-Dihydroxy-4,5-dihydrobenzo[a]pyrene	1.5
1-Hydroxybenzo[a]pyrene	2.2
3-Hydroxybenzo[a]pyrene	5.9

Animal husbandry conditions: after acclimation period of 2 weeks

Stocking rate (kg/m^3)	10.9 ± 2.0
Water temperature (°C)	13.9 ± 0.9
pH	8.0 ± 0.1
NH_4 (mg/l)	0.0 ± 0.0
NO_2 (mg/l)	0.1 ± 0.08
NO_3 (mg/l)	53.6 ± 14.6
$CaCO_3$ (mg/l)	167.3 ± 14.7
Salinity (‰)	0.31 ± 0.02

Note:
For thawing of fish (rainbow trout) hepatocytes please follow the manual "Thawing of Primary Cryopreserved Hepatocytes".

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.

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