PRIMACYT
Cell Culture Technology

RTHCS Cryopreserved Rainbow Trout Hepatocytes for Suspension Assays
Cell Specification

Lot RTH180212-1 Pool	Batch Release: June 1, 2018 – Updated Sept 17, 2020

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 = 1
Post-thaw viability: 89.4 %
Post-thaw yield per vial: 3.54 x 10^6 cells
Recovery: 23.6 %

Viability test on 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 = 1

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

Determination of CYP activities in suspension (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):

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

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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 Fish Hepatocytes".

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