

**RTHCS Cryopreserved Rainbow Trout Hepatocytes for Suspension Assays
Cell Specification – Certificate of Analysis (CoA)**

Lot RTH200415 Pool Batch Release: November 26, 2020

Species: Rainbow trout (<i>Oncorhynchus mykiss</i>) Strain: Christophersen, Bornhoeved Supplier: Fish farm Volker Christophersen Acclimation temperature: 10.0 ± 2.8 °C Age: approx. 2 years	Number and gender of animals: 4 females and 2 males (sexual immature) All animals were kept under controlled environmental conditions at "Aquaristikshop" in Schwerin.
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Animal characteristics:

Donor	1	2	3	4	5	6
Fish weight (g)	466	453	428	469	475	404
Liver weight (g)	6	5	4	4	5	3
Gonad weight (g)	0.85	0.17	0.15	1.02	0.70	0.68
GSI (gonad weight/fish weight)	0.18	0.04	0.04	0.22	0.15	0.17

GSI = Gonadosomatic index

Cryopreservation: Date: April 15, 2020 Amount per vial: 15.0 x 10 ⁶ cells	Thawing: n=1 Post-thaw viability: 99.5 % Post-thaw yield per vial: 11.1 x 10 ⁶ cells Recovery: 74 %
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Viability test on orbital shaker (Eppendorf Thermomixer C, 1000 rpm at 14 °C with 0.5 x 10⁶ cells in 0.5 ml L-15 medium with 5 % FCS):

Time (h)	0	1	2	3	4	5
Viability (%)	99.5	98.0	98.3	97.1	98.5	98.3

Determination of CYP activities in suspension (Eppendorf Thermomixer C, 1000 rpm at 14 °C with 0.5 x 10⁶ 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 ± 0.4
Bupropion-hydroxylase	3.5 ± 0.2
Midazolam 1'-hydroxylase	10.0 ± 0.8
UDP-Glucuronosyltransferase	35.2 ± 1.9
Sulfotransferase	12.3 ± 0.5

Animal husbandry conditions after acclimation period of 2 weeks:

Stocking rate (kg/m ³)	2.2 ± 1.1
Water temperature (°C)	11.4 ± 0.6
pH	8.0 ± 0.1
NH ₄ (mg/l)	3.0 ± 1.0
NO ₂ (mg/l)	0.6 ± 0.7
NO ₃ (mg/l)	4.9 ± 1.6
Carbonate hardness [°KH]	9.6 ± 0.6
Salinity (‰)	0.2 ± 0.1
Conductivity [µS/cm]	553.4 ± 6.3

Note: For thawing of fish (rainbow trout) hepatocytes please follow the respective conditions in our manual "Thawing and Culturing of Cryopreserved Primary Hepatocytes in 2D and Suspension".

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. Thiede

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