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Cell Culture Technology

ASHCS Cryopreserved Atlantic Salmon Hepatocytes for Suspension Assays
Cell Specification – Certificate of Analysis (CoA)

Lot ASH200925-3 Pool	Batch Release: November 27, 2020

Species: Atlantic salmon (Salmo salar)
Supplier: Fish farm Danish Salmon, Hirtshals, DK
Age: approx. 1 year

Number and gender of animals: 3, female sexually immature

All animals were kept under controlled environmental conditions at "Aquaristikshop" in Schwerin.

Animal characteristics:

Donor	1	2	3
Fish weight (g)	229	230	220
Liver weight (g)	7.5	5.2	6.5
Gonad weight (g)	0.35	0.39	0.45
GSI (gonad weight/fish weight)	0.15	0.17	0.21

GSI = Gonadosomatic index

Cryopreservation:
Date: Sep 25, 2020
Amount per vial: 10.3 x 10^6 cells

Thawing: n=2
Post-thaw viability: 96.5 ± 1.6 %
Post-thaw yield per vial: 10.5 ± 1.0 x 10^6 cells
Recovery: 102 ± 9 %

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= 2

Time (h)	0	1	2	3	4	5	24
Viability (%)	96.5 ± 1.6	96.3 ± 0.7	96.1 ± 0.8	93.4 ± 1.8	94.9 ± 0.3	96.5 ± 1.4	90.2 ± 6.4

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	1.6 ± 0.3
Bupropion-hydroxylase	5.6 ± 0.9
Midazolam 1'-hydroxylase	12.7 ± 2.8
UDP-Glucuronosyltransferase	2.9 ± 0.4
Sulfotransferase	12.0 ± 1.4

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Animal husbandry conditions:

Water temperature (°C)	13.5 ± 0.3
pH	8.2 ± 0.1
NH_4 (mg/l)	1.9 ± 1.2
NO_2 (mg/l)	0.5 ± 0.4
NO_3 (mg/l)	1.5 ± 0.6
Carbonate hardness [°KH]	18.8 ± 0.5
Salinity (‰)	32.3 ± 1.2
Conductivity [µS/cm]	49,500 ± 1,731

Note: For thawing of fish (Atlantic salmon) 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 LN_2.

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

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