

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

Lot ASH200924 Pool

Batch Release: October 19, 2020 – Updated Nov 27, 2020

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

Number and gender of animals: 8, female
sexually immature

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

Animal characteristics:

Donor	1	2	3	4	5	6	7	8
Fish weight (g)	220	289	236	290	331	273	275	280
Liver weight (g)	5.0	8.6	6.7	6.4	8.7	6.1	7.5	8.6
Gonad weight (g)	0.35	0.46	0.37	0.35	0.53	0.38	0.39	0.44
GSI (gonad weight/fish weight)	0.16	0.16	0.15	0.12	0.16	0.14	0.14	0.16

GSI = Gonadosomatic index

Cryopreservation:
Date: Sep 24, 2020
Amount per vial: 15 x 10⁶ cells

Thawing: n=1
Post-thaw viability: 93.6 %
Post-thaw yield per vial: 7.0 x 10⁶ cells
Recovery: 47 %

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	24
Viability [%]	93.6	98.2	98.2	97.9	97.9	98.1	97.4

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	1.6 ± 0.2
Bupropion-hydroxylase	9.4 ± 0.8
Midazolam 1'-hydroxylase	14.3 ± 2.2
UDP-Glucuronosyltransferase	5.3 ± 0.4
Sulfotransferase	24.7 ± 3.0

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 LN₂.

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

Issued by: M. Thiede

Checked by: C. Garve