

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

Lot ASH200923-1 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: 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]	280	324	314
Liver weight [g]	5.9	7.3	6.5
Gonad weight [g]	0.60	0.43	0.48
GSI (gonad weight/fish weight)	0.21	0.13	0.15

GSI = Gonadosomatic index

Cryopreservation:
Date: Sep 23, 2020
Amount per vial: 15×10^6 cells

Thawing: n=1
Post-thaw viability: 96.1 %
Post-thaw yield per vial: 5.8×10^6 cells
Recovery: 39 %

Viability test on orbital shaker (Eppendorf Thermomixer C, 1000 rpm at 14 °C with 0.5×10^6 cells in 0.5 ml L-15 medium with 5 % FCS):

Time [h]	0	1	2	3	4	5	24
Viability [%]	96.1	97.7	97.8	97.9	98.1	98.0	96.7

Determination of CYP activities in suspension (Eppendorf Thermomixer C, 1000 rpm at 14 °C with 0.5×10^6 cells in 0.5 ml L-15 medium with 5 % FCS):

Assay	Enzyme activities (pmol/min*mg protein)
	mean ± SD
Phenacetin-O-deethylase	2.1 ± 0.2
Bupropion-hydroxylase	11.5 ± 1.0
Midazolam 1'-hydroxylase	11.1 ± 1.5
UDP-Glucuronosyltransferase	4.7 ± 0.1
Sulfotransferase	30.2 ± 3.1

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