

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

Lot ASH200925-1 Pool

Batch Release: October 23, 2020 – Updated Nov 27, 2020

Species: Atlantic salmon (<i>Salmo salar</i>) Supplier: Fish farm Danish Salmon, Hirtshals, DK Age: approx. 1 year	Number and gender of animals: 5, female sexually 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
Fish weight (g)	277	307	319	228	184
Liver weight (g)	7.1	7.3	6.5	5.3	4.4
Gonad weight (g)	0.42	0.58	0.50	0.40	0.45
GSI (gonad weight/fish weight)	0.15	0.19	0.16	0.17	0.24

GSI = Gonadosomatic index

Cryopreservation: Date: Sep 25, 2020 Amount per vial: 15 x 10 ⁶ cells	Thawing: n=1 Post-thaw viability: 97.7 % Post-thaw yield per vial: 6.9 x 10 ⁶ cells Recovery: 46 %
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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	24
Viability (%)	97.7	97.3	97.8	97.6	98.0	97.6	97.6

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	1.4 ± 0.3
Bupropion-hydroxylase	6.5 ± 0.7
Midazolam 1'-hydroxylase	15.6 ± 1.2
UDP-Glucuronosyltransferase	12.8 ± 0.2
Sulfotransferase	27.6 ± 1.6

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

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