

**ASHCS Cryopreserved Atlantic Salmon Hepatocytes for Suspension Assays
Cell Specification**

Lot ASH180314 Pool

Batch Release: April 19, 2018

Species: Atlantic salmon (<i>Salmo salar</i>) Acclimation temperature: 14.8 ± 0.4 °C sexually immature Age: approx. 1-1.5 years	Number and gender of animals: 3 female
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Animal characteristics:

Donor	1	2	3
Fish weight (g)	334	258	247
Liver weight (g)	2.5	2.0	2.5
Gonad weight (g)	0.69	0.65	0.56
GSI (gonad weight/fish weight)	0.21	0.25	0.23

GSI = Gonadosomatic index

Cryopreservation:

Date: March 14, 2018
Amount per vial: 10.1 x 10⁶ cells

Thawing: n = 2

Post-thaw viability: 98.9 %
Post-thaw yield per vial: 3.98 x 10⁶ cells
Recovery: 39 %

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):

n = 2

Time (h)	0	0.5	1	1.5	2	3	4	5
Viability (%)	98.9	97.8	96.7	97.2	97.2	98.1	96.8	96.5

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	1.9 ± 0.1
Bupropion-hydroxylase	5.1 ± 0.4
Diclofenac 4'-hydroxylase	3.2 ± 1.0
Bufuralol 1'-hydroxylase	4.7 ± 0.4
Midazolam 1'-hydroxylase	10.3 ± 0.7

Animal husbandry conditions during 4 weeks of acclimatisation:

Water temperature (°C)	14.8 ± 0.4
pH	8.3 ± 0.0
NH ₄ (mg/ml)	0.0 ± 0.0
NO ₂ (mg/ml)	0.0 ± 0.0
NO ₃ (mg/ml)	48.3 ± 1.2
CaCO ₃ (mg/l)	106.8 ± 0.0
Salinity (‰)	30 ± 0.0

Feeding: Twice a day, ad libitum "Aller Silver 4,5"

Note:

For thawing of fish (Atlantic salmon) hepatocytes please follow the manual "Thawing of Primary Cryopreserved Fish Hepatocytes".

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