

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

Lot ASH180312 Pool	Batch Release: April 19, 2018
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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 (2 male, 1 female)
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Animal characteristics:

Donor	1	2	3
Fish weight (g)	404	256	314
Liver weight (g)	5.5	2.5	3.0
Gonad weight (g)	0.05	0.09	0.56
GSI (gonad weight/fish weight)	0.01	0.04	0.18

GSI = Gonadosomatic index

Cryopreservation: Date: March 12, 2018 Amount per vial: 10.0 x 10 ⁶ cells	Thawing: n = 2 Post-thaw viability: 97.7 % Post-thaw yield per vial: 3.7 x 10 ⁶ cells Recovery: 37 %
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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):
n = 2

Time (h)	0	0.5	1	1.5	2	3	4	5
Viability (%)	97.7	97.7	96.6	97.7	98.6	98.5	98.3	97.3

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	0.5 ± 0.4
Bupropion-hydroxylase	4.4 ± 0.4
Diclofenac 4'-hydroxylase	4.3 ± 1.0
Bufuralol 1'-hydroxylase	3.4 ± 0.3
Midazolam 1'-hydroxylase	13.4 ± 1.3

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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