

**RTHCS Cryopreserved Rainbow Trout Hepatocytes for Suspension Assays**  
**Cell Specification**

Lot RTH180221 Pool

Batch Release: June 1, 2018 – Updated Sept 17, 2020

Species: Rainbow trout (*Oncorhynchus mykiss*)  
Strain: Christophersen, Bornhoeved  
Supplier: Fish breeding Christophersen  
Acclimation temperature:  $13.2 \pm 1.3$  °C  
Age: approx. 2 years

Number and gender of animals: 2 female and 2 male  
sexual immature

All animals were kept under controlled environmental conditions at Fraunhofer EMB in Lübeck.

**Animal characteristics:**

Donor	1	2	3	4
Fish weight (g)	359	444	354	375
Liver weight (g)	6.64	5.18	5.75	5.45
Gonad weight (g)	0.40	0.87	0.14	0.17
GSI (gonad weight/fish weight)	0.11	0.20	0.04	0.05

GSI = Gonadosomatic index

**Cryopreservation:**

Date: February 21, 2018  
Amount per vial:  $10.1 \times 10^6$  cells

Thawing: n = 1

Post-thaw viability: 98.0 %  
Post-thaw yield per vial:  $5.82 \times 10^6$  cells  
Recovery: 58.2 %

Viability test on orbital shaker (Eppendorf Thermomixer C, 1000 rpm at 14 °C with  $0.5 \times 10^6$  cells in 0.5 ml L-15 medium with 5 % FCS):  
n = 1

Time (h)	0	0.5	1	2	3
Viability (%)	98.0	98.1	97.9	98.1	97.9

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

Assay	Enzyme activities (pmol/min*mg protein) mean $\pm$ SD
Phenacetin-O-deethylase	$4.3 \pm 0.1$
Bupropion-hydroxylase	$1.6 \pm 0.1$
Diclofenac 4'-hydroxylase	$4.3 \pm 0.9$
Bufuralol 1'-hydroxylase	$1.5 \pm 0.0$
Midazolam 1'-hydroxylase	$5.5 \pm 0.8$
UDP-Glucuronosyltransferase	$22.1 \pm 1.1$
Sulfotransferase	$11.2 \pm 1.4$

Animal husbandry conditions after acclimation period of 2 weeks:

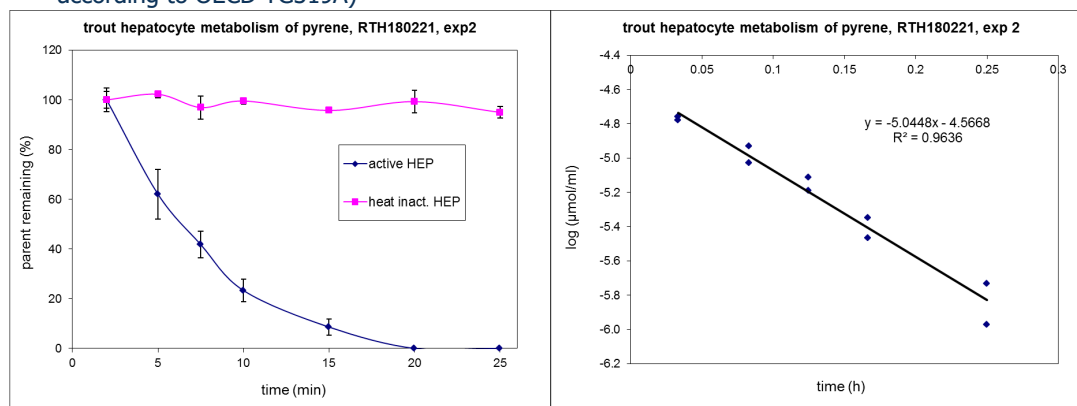
Stocking rate (kg/m <sup>3</sup> )	10.9 ± 2.0
Water temperature (°C)	13.9 ± 0.9
pH	8.0 ± 0.1
NH <sub>4</sub> (mg/l)	0.0 ± 0.0
NO <sub>2</sub> (mg/l)	0.1 ± 0.08
NO <sub>3</sub> (mg/l)	53.6 ± 14.6
CaCO <sub>3</sub> (mg/l)	167.3 ± 14.7
Salinity (‰)	0.31 ± 0.02

**Pyrene Depletion** (Data kindly provided by Lu Hostettler and Heike Laue, Givaudan, Dübendorf, Switzerland)

In vitro intrinsic clearance of Pyrene was determined according to OECD TG 319A. Hepatocytes were thawed according to the protocol provided by Primacyt. L15 medium w/o phenol red from Gibco was used and prepared according to OECD TG 319A (pH adjusted to 7.80 at 11 °C). Incubation was performed according to OECD TG 319A (in brief: 25 nM Pyrene, 25 min, 11 °C, 400 rpm, 2 x 10<sup>6</sup> cells/ml, 1 ml, negative control: heat inactivated trout hepatocytes, analysis of pyrene concentration by GC-MS, with anthracene as internal standard for quantification.

**Results:**

- Depletion rate constant (k): **11.60 h<sup>-1</sup>**
- R<sup>2</sup>: 0.9636
- **in vitro intrinsic clearance: 7.20 mL \* h<sup>-1</sup> \* 10<sup>6</sup> cells<sup>-1</sup>** (calculated with the measured cell concentration, according to OECD TG319A)



**Left panel:** % parent remaining with initial pyrene concentration set to 100%, **right panel:** Pyrene depletion curve (log plot)

Note: For thawing of fish (rainbow trout) hepatocytes please follow the manual "Thawing of Primary Cryopreserved Fish Hepatocytes". This product should be considered as potential biohazard. Only intended for *in vitro* use.

**Store at -150 °C**

Datasheet updated December 6, 2018

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