PRIMACYT
Cell Culture Technology

RTHCS Cryopreserved Rainbow Trout Hepatocytes for Suspension Assays
Cell Specification – Certificate of Analysis (CoA)

Lot RTH200415 Pool	Batch Release: Nov 26, 2020 – Last Update: Dec 14, 2022

Species: Rainbow trout (Oncorhynchus mykiss)
Strain: Christophersen, Bornhoeved
Supplier: Fish farm Volker Christophersen
Acclimation temperature: 10.0 ± 2.8 °C
Age: approx. 2 years

Number and gender of animals: 4 females and 2 males (sexual immature)

All animals were kept under controlled environmental conditions at "Aquaristikshop" in Schwerin.

Animal characteristics:

Donor	1	2	3	4	5	6
Fish weight (g)	466	453	428	469	475	404
Liver weight (g)	6	5	4	4	5	3
Gonad weight (g)	0.85	0.17	0.15	1.02	0.70	0.68
GSI (gonad weight/fish weight)	0.18	0.04	0.04	0.22	0.15	0.17

GSI = Gonadosomatic index

Cryopreservation:
Date: April 15, 2020
Amount per vial: 15.0 x 10^6 cells

Thawing: n = 3
Post-thaw viability: 98.6 ± 1.2 %
Post-thaw yield per vial: 8.1 ± 2.2 x 10^6 cells
Recovery: 61 %

Viability test: orbital shaker (Eppendorf Thermomixer C, 1000 rpm at 14 °C with 0.5 x 10^6 cells in 0.5 ml L-15 medium with 5 % FCS): n = 2

Time (h)	0	1	2	3	4	5	15	24
Viability (%)	99.5	98.0	98.3	97.1	98.5	98.3	99.1	99.3

Phase I and Phase II metabolism: Determination of enzymatic activities in suspension

Assay	**Enzyme activities (pmol/min*mg protein)** mean ± SD
Phenacetin-O-deethylase	2.6 ± 0.4
Bupropion-hydroxylase	3.5 ± 0.2
Midazolam 1'-hydroxylase	10.0 ± 0.8
UDP-Glucuronosyltransferase	35.2 ± 1.9
Sulfotransferase	12.3 ± 0.5

Enzyme activity assays were performed at PRIMACYT GmbH. The assays were conducted with 0.5 x 10^6 cells in 0.5 ml L-15 medium with 5 % FCS at 14 °C and 1.000 rpm using an Eppendorf Thermomixer C. Values for enzyme activities were determined at a single substrate concentration and are mean ± standard deviation of three determinations. Metabolite formation was determined with validated LC-MS/MS methods by a GLP certified external service provider.

E-mail: info@primacyt.com, Tel.: +49 (0) 385-3993-600, Fax: +49 (0) 385-3993-602

PRIMACYT
Cell Culture Technology

Determination of Polycyclic Aromatic Hydrocarbons (PAH) metabolism:

Incubation of trout hepatocytes with 20 µM Benzo[a]pyrene for 24 h. Determination of Benzo[a]pyrene metabolites by HPLC-FLD in cell culture medium after treatment with ß-Glucuronidase/Arylsulfatase. Chemical analysis of Benzo[a]pyrene metabolites was performed by Biochemisches Institut für Umweltcarcinogene, Prof. Dr. Gernot Grimmer Stiftung, Großhansdorf, Germany.

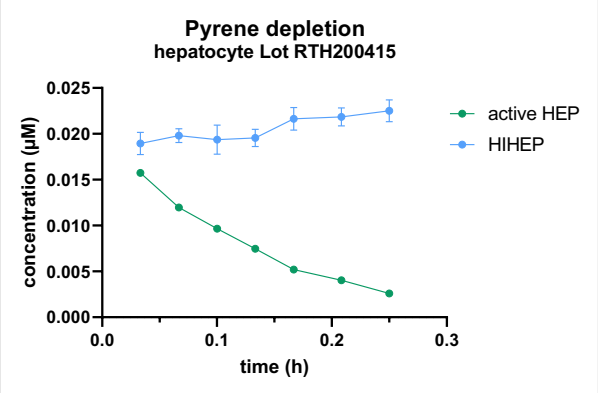
Metabolite	ng/ml
trans-7,8-Dihydroxy-4,5-dihydrobenzo[a]pyrene	1.9
1-Hydroxybenzo[a]pyrene	4.1
3-Hydroxybenzo[a]pyrene	8.4

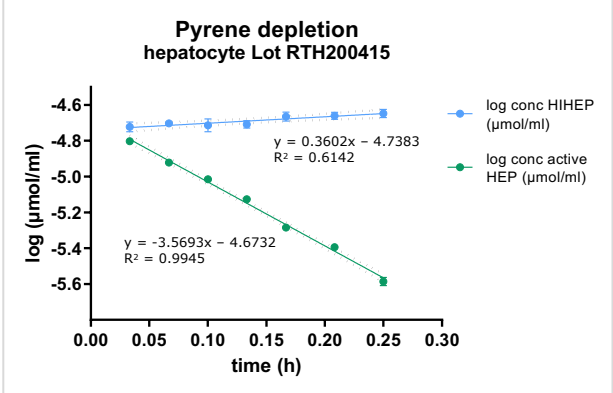
Pyrene Depletion (Data kindly provided by Lu Hostettler and Heike Laue, Givaudan, Kemptthal, Switzerland)

In vitro intrinsic clearance of Pyrene was determined according to OECD TG 319A. Hepatocytes were thawed according to the protocol provided by Primacyt. Thawing (HTM) and washing (HWM) medium provided by Primacyt were used (Lot HTM220302 and HWM220302). Cells were resuspended at a final concentration of 2 x 10^6 cells/mL in L-15 medium (Gibco, 21083-027, Lot 2323515, pH 7.8 at 11 °C). Incubation was performed according to OECD TG 319A (in brief: 25 nM Pyrene, 15 min, 11 °C, 400 rpm, 2 x 10^6 cells/ml, in single vials using 1 ml suspension, duplicates, negative control: heat inactivated trout hepatocytes (HIHEP, lot from ring trial), analysis of pyrene concentration by GC-MS, with anthracene as internal standard for quantification.)

Results:

- Depletion rate constant (k): **8.21 h^{-1}**
- R^2: **0.9945**
- in vitro intrinsic clearance: **5.41 $mL*h^{-1}*10^6$ $cells^{-1}$** (calculated with the measured cell concentration, according to OECD TG319A)





Left panel: Measured Pyrene concentration over time for active and heat inactivated trout hepatocytes, **right panel:** Pyrene depletion curve (log plot)

Animal husbandry conditions: after acclimation period of 2 weeks:

Stocking rate (kg/m^3)	2.2 ± 1.1
Water temperature (°C)	11.4 ± 0.6
pH	8.0 ± 0.1
NH_4 (mg/l)	3.0 ± 1.0
NO_2 (mg/l)	0.6 ± 0.7
NO_3 (mg/l)	4.9 ± 1.6
Carbonate hardness [°KH]	9.6 ± 0.6
Salinity (‰)	0.2 ± 0.1
Conductivity [µS/cm]	553.4 ± 6.3

Note: For thawing of fish (rainbow trout) 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 LN_2.

This product should be considered as potential biohazard. Only intended for *in vitro* use.

Issued by: M. Thiede Checked by: C. Garve

Updated by: D. Runge, A. Ullrich Checked by: K. Damrau