

Species: Rainbow trout (Oncorhynchus mykiss) strain: Christophersen, Bornhoeved supplier: Fish breeding Christophersen kcclimation temperature: $13.2 \pm 1.3$ °C (ge: approx. 2 yearsNumber and gender of animals: 1 female, sexual immatureAll animals were kept under controlled environmental conditions at Fraunhofer E Lübeck.All animals were kept under controlled environmental conditions at Fraunhofer E Lübeck.Animal characteristics: Donor123Donor123Fish weight (g)6.335.353.68Gonad weight/(g)-0.140.46GSI (gonad weight/fish weight)-0.040.14SI = Gonadosomatic indexThawing: n = 3Cryopreservation: Date: Date: S ml L-15 medium with 5 % FCS): n = 3Thawing: n = 3Time (h)00.511.523451!Viability (%)89.489.492.788.096.495.789.796.298.3Phase I and Phase II metabolism: Diclofena c4'-hydroxylase2.2 ± 0.11.52.2 ± 0.11.52.2 ± 0.1Diclofena c4'-hydroxylase1.2 ± 0.1Midazolam 1'-hydroxylase2.2 ± 0.11.5 ± 1.3 ± 1.31.5 ± 1.3 ± 1.31.5 ± 1.3 ± 1.3Diclofena c4'-hydroxylase1.2 ± 0.1Midazolam 1'-hydroxylase1.2 ± 0.11.4 ± 1.31.4 ± 1.31.5 ± 1.3 ± 1.3Diclofena c4'-hydroxylase1.2 ± 0.1Midazolam 1'-hydroxylase1.2 ± 0.11.4 ± 1.31.1 ± 1.3Diclofena c4'-hydroxylase1.2	, 2 male								Cell Specification Lot RTH180212-1 Pool					
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Supplier: Fish breeding Christophersen Acclimation temperature: $13.2 \pm 1.3$ °C All animals were kept under controlled environmental conditions at Fraunhofer E Lübeck.   Age: approx. 2 years I 2 3 I 2 3 I 2 3   Animal characteristics: Donor   Donor 1 2 3 I 2 3   Fish weight (g) 6.33 5.35 3.68   Gonad weight (g) - 0.14 0.46   GSI (gonad weight/fish weight) - 0.04 0.14   SSI = Gonadosomatic index Post-thaw viability: 85.6 $\pm 2.7$ % Post-thaw viability: 85.6 $\pm 2.7$ % Post-thaw yield per vial: $2.5 \pm 0.8 \times 10^6$ cells   Cryopreservation: Date: February 12, 2018 Post-thaw yield per vial: $2.5 \pm 0.8 \times 10^6$ cells   Miability test: Orbital shaker (Eppendorf Thermomixer C, 1000 rpm at 14 °C with 0.5 x 10 <sup>6</sup> cell   Dis ml L-15 medium with 5 % FCS): n = 3   Time (h) 0 0.5 1 1.5 2 3 4 5 11   Viability (%) 89.4 92.7 88.0 96.4 95.7 89.7 96.2 98.7   Phenacet In Orbethylase 2.2 $\pm 0.1$ mean $\pm$ SD mean $\pm$ SD Phenacetin/Ordeethylase 2.6 $\pm 0.9$ Bupropion-hydroxylase 2.2 $\pm 0.1$ Diclofenac 4'-hydroxylase 1.2 $\pm 0.1$ <	MB in	Number and gender of animals: 1 female, 2 male					Species: Rainbow trout (Oncorhynchus mykiss)							
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ml L-15 medium with 5 % FCS at 14 °C and 1.000 rpm using an Eppendorf Thermomixer C. Values for enz activities were determined at a single substrate concentration and are mean ± standard deviation of three								0 10111 US		5 dl 14 °C		1E modium w		

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## 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 β-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	1.5
1-Hydroxybenzo[a]pyrene	2.2
3-Hydroxybenzo[a]pyrene	5.9

## **Animal husbandry conditions:** after acclimation period of 2 weeks

Stocking rate (kg/m <sup>3</sup> )	$10.9 \pm 2.0$
Water temperature (°C)	$13.9 \pm 0.9$
рН	$8.0 \pm 0.1$
NH <sub>4</sub> (mg/l)	$0.0 \pm 0.0$
NO <sub>2</sub> (mg/l)	$0.1 \pm 0.08$
NO₃ (mg/l)	53.6 ± 14.6
CaCO <sub>3</sub> (mg/l)	167.3 ± 14.7
Salinity (‰)	$0.31 \pm 0.02$

## Note:

For thawing of fish (rainbow trout) hepatocytes please follow the manual "Thawing of Primary Cryopreserved Hepatocytes".

## Store at -150 °C or in the vapour phase of LN<sub>2</sub>.

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

Issued by: M. Thiede	Checked by: A. Ullrich
Updated by: A. Ullrich	Checked by: K.Damrau