

CCHCS Cryopreserved Common Carp Hepatocytes for Suspension Assays
Cell Specification – Certificate of Analysis (CoA)

Lot CCH200512 Pool

Batch Release: November 27, 2020

Species: Common Carp (*Cyprinus carpio*)
Supplier: Fish farm Lewitz Fisch Hermann Stahl,
Neustadt-Glewe
Acclimation temperature: 13.7 ± 0.7 °C
Age: approx. 1-2 years

Number and gender of animals: 2 female and 1
male (sexually immature)

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

Animal characteristics:

| Donor | 1 | 2 | 3 |
|--------------------------------|------|------|------|
| Fish weight [g] | 276 | 376 | 370 |
| Gonad weight [g] | 0.50 | 1.73 | 1.14 |
| GSI (gonad weight/fish weight) | 0.18 | 0.46 | 0.31 |

GSI = Gonadosomatic index

Cryopreservation:
Date: May 12, 2020
Amount per vial: 7×10^6 cells

Thawing: n=2
Post-thaw viability: 96.3 ± 1.0 %
Post-thaw yield per vial: $2.6 \pm 0.04 \times 10^6$ cells
Recovery: 37 ± 0.5 %

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

| Time [h] | 0 | 1 | 2 | 3 | 4 | 5 |
|---------------|------|------|------|------|------|------|
| Viability [%] | 96.3 | 94.7 | 96.2 | 92.7 | 97.8 | 96.6 |

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

| Assay | Enzyme activities (pmol/min*mg protein) mean \pm SD |
|--------------------------|--|
| Phenacetin-O-deethylase | 2.4 ± 0.6 |
| Bupropion-hydroxylase | 1.8 ± 0.3 |
| Midazolam 1'-hydroxylase | 4.8 ± 0.9 |

Animal husbandry conditions after acclimation period of 2 weeks:

| | |
|--------------------------|-------------|
| Water temperature [°C] | 14.1 ± 1.0 |
| pH | 8.1 ± 0.1 |
| NH ₄ [mg/L] | 0.05 ± 0.06 |
| NO ₂ [mg/L] | 0.05 ± 0.03 |
| NO ₃ [mg/L] | 12.0 ± 1.6 |
| Carbonate hardness [°KH] | 8.1 ± 0.3 |
| Salinity [‰] | 0.2 ± 0.0 |
| Conductivity [µS/cm] | 568 ± 5.0 |

Note: For thawing of fish 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 LN₂.

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

Issued by: M. Thiede

Checked by: C. Garve