

## RH-35 Cells | 305210

## General information

## Description

The H4-II-E (also referred to as RH-35) cell line is a derivative of the Reuber H-35 rat hepatoma. This cell line originated from a liver tumor induced in a male ACI rat by exposure to the chemical carcinogen N-2-fluorenyldiacetamide. When transplanted into ACI rats, H4-II-E cells form rapidly growing tumors with histological features characteristic of poorly differentiated hepatomas. They are particularly sensitive to the induction of aryl hydrocarbon hydroxylase (AHH) activity, making them a robust system for studying enzymatic responses to polycyclic aromatic hydrocarbons and dioxin-like compounds.

H4-II-E cells also serve as a model for studying cellular responses to carcinogens and radiation, given their clonogenicity and the ability to assay long-term cell survival post-treatment. Their application extends to exploring the mechanisms of enzyme induction, xenobiotic metabolism, and liver-specific toxicology. These attributes make H4-II-E an invaluable tool in cancer research and toxicological screening.

**Organism** Rat

**Tissue** Liver

**Disease** Rat hepatocellular carcinoma

**Synonyms** H4II, H-35tc2, Reuber-H-35 hepatoma tissue culture 2, Reuber H-35 tc2, Reuber H35 tc2, H-35 Reuber tc2, H35 Reuber tc2, RH-35 tc2, RH35 tc2, H-35 tc2, H35 tc2

## Characteristics

**Breed/Subspecies** AxC

**Gender** Male

**Morphology** Epithelial

**Growth properties** Adherent

## Regulatory Data

**Citation** RH-35 (Cytion catalog number 305210)

**Biosafety level** 1

**NCBI\_TaxID** 10116

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CellosaurusAccession CVCL\_4623

**Biomolecular Data****Handling**

<b>Culture Medium</b>	Ham's F12, w: 1.0 mM stable Glutamine, w: 1.0 mM Sodium pyruvate, w: 1.1 g/L NaHCO <sub>3</sub> (Cytion article number 820600a)
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<b>Supplements</b>	Supplement the medium with 10% FBS
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<b>Dissociation Reagent</b>	Accutase
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<b>Subculturing</b>	Remove the old medium from the adherent cells and wash them with PBS that lacks calcium and magnesium. For T25 flasks, use 3-5 ml of PBS, and for T75 flasks, use 5-10 ml. Then, cover the cells completely with Accutase, using 1-2 ml for T25 flasks and 2.5 ml for T75 flasks. Let the cells incubate at room temperature for 8-10 minutes to detach them. After incubation, gently mix the cells with 10 ml of medium to resuspend them, then centrifuge at 300xg for 3 minutes. Discard the supernatant, resuspend the cells in fresh medium, and transfer them into new flasks that already contain fresh medium.
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<b>Fluid renewal</b>	2 to 3 times per week
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<b>Freeze medium</b>	As a cryopreservation medium, we use complete growth medium (including FBS) + 10% DMSO for adequate post-thaw viability, or CM-1 (Cytion catalog number 800100), which includes optimized osmoprotectants and metabolic stabilizers to enhance recovery and reduce cryo-induced stress.
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### Thawing and Culturing Cells

1. Confirm that the vial remains deeply frozen upon delivery, as cells are shipped on dry ice to maintain optimal temperatures during transit.
2. Upon receipt, either store the cryovial immediately at temperatures below  $-150^{\circ}\text{C}$  to ensure the preservation of cellular integrity, or proceed to step 3 if immediate culturing is required.
3. For immediate culturing, swiftly thaw the vial by immersing it in a  $37^{\circ}\text{C}$  water bath with clean water and an antimicrobial agent, agitating gently for 40-60 seconds until a small ice clump remains.
4. Perform all subsequent steps under sterile conditions in a flow hood, disinfecting the cryovial with 70% ethanol before opening.
5. Carefully open the disinfected vial and transfer the cell suspension into a 15 ml centrifuge tube containing 8 ml of room-temperature culture medium, mixing gently.
6. Centrifuge the mixture at  $300 \times g$  for 3 minutes to separate the cells and carefully discard the supernatant containing residual freezing medium.
7. Gently resuspend the cell pellet in 10 ml of fresh culture medium. For adherent cells, divide the suspension between two T25 culture flasks; for suspension cultures, transfer all the medium into one T25 flask to promote effective cell interaction and growth.
8. Adhere to established subculture protocols for continued growth and maintenance of the cell line, ensuring reliable experimental outcomes.

### Incubation Atmosphere

$37^{\circ}\text{C}$ , 5%  $\text{CO}_2$ , humidified atmosphere.

### Flask Coating

None

### Shipping Conditions

Cryopreserved cell lines are shipped on dry ice in validated, insulated packaging with sufficient refrigerant to maintain approximately  $-78^{\circ}\text{C}$  throughout transit. On receipt, inspect the container immediately and transfer vials without delay to appropriate storage.

### Storage Conditions

For long-term preservation, place vials in vapor-phase liquid nitrogen at about  $-150$  to  $-196^{\circ}\text{C}$ . Storage at  $-80^{\circ}\text{C}$  is acceptable only as a short interim step before transfer to liquid nitrogen.

## Quality Control & Molecular Analysis

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**Sterility**

Mycoplasma contamination is excluded using both PCR-based assays and luminescence-based mycoplasma detection methods.

To ensure there is no bacterial, fungal, or yeast contamination, cell cultures are subjected to daily visual inspections.