**Product sheet**

# HEp-2 Cells | 300397

## General information

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| **Description** | The HEp-2 cell line, originally believed to be derived from laryngeal cancer cells, was later identified through DNA fingerprinting and the presence of HeLa marker chromosomes as being contaminated with HeLa cells, a cell line that was derived from cervical cancer.<br><br>Despite this, the HEp-2 cell line remains extensively utilized in indirect immunofluorescence to detect antinuclear antibodies (ANAs), which are key in diagnosing conditions like systemic lupus erythematosus and systemic sclerosis. The indirect immunofluorescence assay (IIFA) using HEp-2 cells, which provides clear positive or negative results, is the standard method for testing antinuclear antibodies. This straightforward approach is crucial for diagnosing and classifying different systemic autoimmune diseases.<br><br>The patterns of autoantibodies observed in indirect immunofluorescence on HEp-2 cells, especially in the context of rheumatology, provide invaluable insights into various rheumatic diseases. Furthermore, the comprehensive review of antigens expressed by HEp-2 human cells under different culture conditions enables the identification of specific ANAs linked to diseases like lupus.<br><br>In conclusion, while the contamination of cell lines like HEp-2 with HeLa cells has prompted concerns in cancer research about the accuracy and reliability of results and their clinical relevance, the utility of Hep-2 in the detection of antinuclear antibodies and its application across various research disciplines underscore its continued importance. The HEp-2 cell line serves as an essential tool in diagnosing and classifying autoimmune diseases, among other applications. |
| **Organism** | Human |
| **Tissue** | Larynx |
| **Disease** | Adenocarcinoma |
| **Applications** | In rheumatology, indirect immunofluorescence using HEp-2 cells plays a crucial role in diagnosing autoimmune diseases, including systemic lupus erythematosus and systemic sclerosis |
| **Synonyms** | Hep-2, HEP-2, HEp-2/HeLa, Hep 2, Hep2, HEp2, HEP2, H.Ep.-2, H.Ep. #2, H.Ep. No. 2, Hep II, Human Epidermoid carcinoma #2, Human Epithelioma-2 |

## Characteristics

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| **Age** | 30 years |
| **Gender** | Female |
| **Ethnicity** | African American |
| **Morphology** | Epithelial-like |

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| **Growth properties** | Monolayer, adherent |

## Regulatory Data

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| **Citation** | HEp-2 (Cytion catalog number 300397) |
| **Biosafety level** | 1 |
| **NCBI_TaxID** | 9606 |
| **CellosaurusAccession** | CVCL_1906 |

## Biomolecular Data

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| **Isoenzymes** | G6PD, A |
| **Reverse transcriptase** | Negative |
| **Products** | Keratin |

## Handling

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| **Culture Medium** | EMEM (MEM Eagle), w: 2 mM L-Glutamine, w: 2.2 g/L NaHCO3, w: EBSS (Cytion article number 820100a) |
| **Supplements** | Supplement the medium with 10% FBS and 1% NEAA |
| **Dissociation Reagent** | Accutase |
| **Subculturing** | 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. |
| **Seeding density** | $1 \times 10^4$ cells/$cm^2$ |

## HEp-2 Cells | 300397

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| **Fluid renewal** | 2 to 3 times per week |
| **Post-Thaw Recovery** | After thawing, plate the cells at 5 x $10^4$ cells/$cm^2$ and allow the cells to recover from the freezing process and to adhere for at least 24 hours. |
| **Freeze medium** | 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. |
| **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.<br>2. Upon receipt, either store the cryovial immediately at temperatures below -150°C to ensure the preservation of cellular integrity, or proceed to step 3 if immediate culturing is required.<br>3. For immediate culturing, swiftly thaw the vial by immersing it in a 37°C water bath with clean water and an antimicrobial agent, agitating gently for 40-60 seconds until a small ice clump remains.<br>4. Perform all subsequent steps under sterile conditions in a flow hood, disinfecting the cryovial with 70% ethanol before opening.<br>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.<br>6. Centrifuge the mixture at 300 x g for 3 minutes to separate the cells and carefully discard the supernatant containing residual freezing medium.<br>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.<br>8. Adhere to established subculture protocols for continued growth and maintenance of the cell line, ensuring reliable experimental outcomes. |
| **Incubation Atmosphere** | 37°C, 5% $CO_2$, humidified atmosphere. |
| **Shipping Conditions** | Cryopreserved cell lines are shipped on dry ice in validated, insulated packaging with sufficient refrigerant to maintain approximately −78 °C throughout transit. On receipt, inspect the container immediately and transfer vials without delay to appropriate storage. |

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| **Storage Conditions** | For long-term preservation, place vials in vapor-phase liquid nitrogen at about −150 to −196 °C. Storage at −80 °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.<br><br>To ensure there is no bacterial, fungal, or yeast contamination, cell cultures are subjected to daily visual inspections. |