**Product sheet**

Cytion

# HTR-8/SVneo Cells | 305221

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| **Description** | HTR-8/SVneo is a human trophoblast cell line derived from the chorionic villi of a first-trimester placenta, specifically from a 6-to-12-week-old embryo. These cells were immortalized by transfecting them with the gene encoding the simian virus 40 (SV40) large T antigen, which extends their lifespan while maintaining characteristics typical of extravillous invasive trophoblasts. This cell line expresses several key markers associated with extravillous trophoblasts, including insulin-like growth factor II (IGF-II), NDOG-5, proliferating cell nuclear antigen (PCNA), and a range of integrins (α1, α3, α5, αv, and β1 subunits, along with the αvβ3/β5 vitronectin receptor). It is negative for macrophage marker 63/D3, endothelial cell marker factor VIII, and α6 and β4 integrin subunits, confirming its trophoblast lineage and distinguishing it from other cell types such as macrophages and endothelial cells.<br><br>HTR-8/SVneo cells are widely used as a model to study trophoblast invasion and placental biology, particularly the epithelial-to-mesenchymal transition (EMT), which is crucial for trophoblasts' invasive behavior during placental development. Research has shown that these cells exhibit a mixed population of epithelial and mesenchymal phenotypes, with the ability to undergo EMT under standard culture conditions. This transition is mediated by TGF-β signaling, which promotes the mesenchymal phenotype, as evidenced by the upregulation of mesenchymal markers such as vimentin and the downregulation of epithelial markers like E-cadherin. This makes HTR-8/SVneo a valuable in vitro model for studying the molecular mechanisms underlying EMT in trophoblasts and its implications in both normal placental development and pregnancy-related disorders.<br><br>Studies have further demonstrated that HTR-8/SVneo cells can form spheroids, which predominantly express epithelial markers. When these spheroids are re-plated in 2D culture, the cells exhibit a shift towards a mesenchymal phenotype, indicating an ongoing EMT process. This cell line's unique properties, including its responsiveness to TGF-β and its mixed epithelial-mesenchymal nature, provide critical insights into the complex cellular dynamics of trophoblast invasion and the regulation of placental development, offering a robust platform for investigating pregnancy-related pathologies such as pre-eclampsia and intrauterine growth restriction. |
| **Organism** | Human |
| **Tissue** | Trophoblast, Placenta |
| **Synonyms** | HTR-8/SV neo, HTR-8/SV-neo, HTR8/SVneo, HTR8svn |

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| **Age** | 6-12 fetal weeks |
| **Gender** | Unspecified |
| **Morphology** | A mixture of epithelial and mesenchymal-like cells |
| **Growth properties** | Adherent |

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| **Citation** | HTR-8/SVneo (Cytion catalog number 305221) |
| **Biosafety level** | 1 |
| **NCBI_TaxID** | 9606 |
| **CellosaurusAccession** | CVCL_7162 |
| **GMO Status** | GMO-S1: This human trophoblast cell line (HTR-8/SVneo) contains an SV40 T-Antigen construct introduced by transfection, enabling immortalization of primary trophoblast cells. The insert is stably integrated. This classification applies only within Germany and may differ elsewhere. |

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| **Viruses** | Simian virus 40 (transfected with pSV3neo plasmid containing the early region of SV40) |

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| **Culture Medium** | RPMI 1640, w: 2.0 mM stable Glutamine, w: 2.0 g/L NaHCO3 (Cytion article number 820700a) |
| **Supplements** | Supplement the medium with 10% FBS |
| **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. |
| **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. |

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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°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°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 x 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°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.

### 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.

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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. |
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