

**AU565 Cells | 305313**

**General information**

**Description**

The AU565 cell line is derived from a human breast carcinoma and is classified as HER2-positive, making it a valuable model for studying HER2-targeted therapies such as trastuzumab (TZM). These cells are widely used to investigate breast cancer behaviors, particularly regarding targeted drug delivery and metastatic processes. Research utilizing AU565 cells has shown that they exhibit significant HER2 expression at the plasma membrane, facilitating studies on the binding efficiency and internalization of anti-HER2 monoclonal antibodies like TZM. AU565 cells display efficient TZM binding at the membrane with subsequent accumulation in intracellular compartments, providing insights into the endocytic and trafficking mechanisms involved in TZM uptake and retention within tumor cells. This unique behavior makes AU565 a distinctive model compared to other HER2-positive cell lines and supports its use in exploring drug efficacy and cell membrane dynamics.

AU565 cells also serve as a model for studying metastatic behavior, specifically transendothelial migration, which is a critical step in cancer metastasis. As a weakly invasive cell line, AU565's ability to migrate across endothelial cell layers is highly dependent on focal adhesion kinase (FAK) signaling, which facilitates interactions with the extracellular matrix and endothelial cells during migration. Inhibiting FAK activity in AU565 cells has been shown to reduce their migration rates, highlighting FAK's role in cell motility and suggesting its potential as a therapeutic target to limit metastatic progression. Additionally, AU565 cells exhibit responses to variations within the tumor microenvironment, such as differences in collagen density, which can impact drug delivery efficacy and resistance. These characteristics make AU565 cells a powerful model for studying HER2-targeted therapies and the influences of the tumor microenvironment on treatment outcomes.

**Organism** Human

**Tissue** Breast

**Disease** Adenocarcinoma

**Metastatic site** Pleural effusion

**Synonyms** AU-565, AU 565

**Characteristics**

**Age** 43 years

**Gender** Female

**Ethnicity** Caucasian

**Morphology** Epithelial-like

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<b>Growth properties</b>	Adherent
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## Regulatory Data

<b>Citation</b>	AU565 (Cytion catalog number 305313)
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<b>Biosafety level</b>	1
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<b>NCBI_TaxID</b>	9606
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<b>CellosaurusAccession</b>	CVCL_1074
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## Biomolecular Data

<b>Receptors expressed</b>	Epidermal growth factor (EGF)
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<b>Oncogenes</b>	Her2/neu+ (overexpressed), her3+, her4+, p53+
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<b>Mutational profile</b>	Mutation: TP53, p.Arg175His (c.524G>A), homozygous
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## Handling

<b>Culture Medium</b>	RPMI 1640, w: 2.0 mM stable Glutamine, w: 2.0 g/L NaHCO <sub>3</sub> (Cytion article number 820700a)
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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>	1 to 2 times per week
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### 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.
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.

### 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

### **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.