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Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

HER2 Knockout MCF7 Cell Line is an MCF7 cell line in which human HER2 (Receptor tyrosine-protein kinase ErbB-2) has been genetically removed using CRISPR/Cas9 genome editing with a lentivirus encoding the CRISPR/Cas9 gene and sgRNA (single guide RNA) targeting human HER2.

This cell line has been validated by sequencing and flow cytometry.

Background

HER2 (human epidermal growth factor receptor 2), also known as erbB-2 or CD340, is a tyrosine kinase receptor of the EFGR family of proteins. There is no known ligand, but it can form homodimers or heterodimers with other HER proteins. Once active, it activates the MAPK (mitogen-activated protein kinase) and PI3K (phosphatidylinositol-3 kinase) signaling pathways resulting in cell cycle progression and cell proliferation. HER2 over-expression is also known to occur in breast, ovarian, stomach, lung adenocarcinoma, aggressive forms of uterine cancer and gastric cancer. In 1990 the FDA approved the use of the monoclonal antibody trastuzumab in breast and stomach cancer. Other strategies to target HER2 that have been approved include ADCs (antibody-drug conjugate) and margetuximab (an anti-HER2 antibody that can alter the Fc-receptor affinity to CD16 and induce cytotoxicity). The use of small molecule tyrosine kinase inhibitors, alone or in combinatory therapy, has shown great promise in the treatment of $HER2^+$ breast cancer (BC). However, resistance to treatment, for instance by mutations on HER2 or upregulation of other HER receptors, has been described. Neratinib, a pan-HER2 inhibitor, was approved in 2017 for early-stage BC, as adjuvant anti-HER2 therapy after trastuzumab treatment. However, side effects limit its use. The development of treatments targeting early-stage cancer, with minimal side effects and resistance development, will bring major benefits to $HER2^+$ oncology patients.

MCF7 is a breast cancer cell line derived from a pleural effusion metastatic site. MCF7 is a hormone receptor positive (HR^+) breast cancer cell line that expresses high levels of both estrogen receptor (ER) and progesterone receptor, with low expression of human epidermal growth factor receptor 2 (HER2). This receptor expression pattern renders MCF7 cell line useful as a model to develop hormone receptor targeting therapeutics and as a target-negative cell line for the development of therapeutics against HER2.

Application

- Study the impact of HER2 knockout.
- Use as a control for developing anti-HER2 CAR-T killing assays.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains >1 x 10^6 cells in 1 ml of Cell Freezing Medium (BPS Bioscience #79796)

Parental Cell Line

MCF7 human breast mammary gland cell line. Adherent epithelial cells.

Mycoplasma Testing

The cell line has been screened to confirm the absence of Mycoplasma species.

Materials Required but Not Supplied

These materials are not supplied with the cell line but are necessary for cell culture and cellular assays. BPS Bioscience's reagents are validated and optimized for use with this cell line and are highly recommended for best results. Media components are provided in the Media Formulations section below.

Media Required for Cell Culture

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Insulin Solution from Bovine Pancreas	Sigma-Aldrich #I0516

Storage Conditions

Cells are shipped in dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a -80°C freezer for long term storage. Contact technical support at support@bpsbioscience.com if the cells are not frozen in dry ice upon arrival.

Media Formulations

For best results, the use of validated and optimized media from BPS Bioscience is *highly recommended*. Other preparations or formulations of media may result in suboptimal performance.

Note: Thaw Media do *not* contain selective antibiotics.
Cells should be grown at 37°C with 5% CO_2. BPS Bioscience's cell lines are stable for at least 10 passages when grown under proper conditions.

Media Required for Cell Culture

Complete Thaw Medium 1*:*

Thaw Medium 1 (BPS Bioscience, #60187) + 10 µg/ml Insulin (Sigma-Aldrich #I0516): MEM medium supplemented with 10% FBS, 1% Penicillin/Streptomycin, 1% Non-Essential amino acids, and 1 mM Na pyruvate + 10 µg/ml Insulin (Sigma-Aldrich #I0516).

Note: The final concentration of 10 µg/ml of Insulin (Sigma-Aldrich #I0516) will need to be added to Thaw Medium 1 for cell culture.

Note: Although the cells do not need to be grown in puromycin to maintain HER2 knockout, the cells are puromycin resistant.

Cell Culture Protocol

Note: MCF7 cells are derived from human material and thus the use of adequate safety precautions is recommended.

Cell Thawing

1. Swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. As soon as the cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire contents of the vial to a tube containing 10 ml of pre-warmed Thaw Medium 1.

   Note: Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

2. Immediately spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Complete Thaw Medium 1.

3. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO_2 incubator.

4. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Complete Thaw Medium 1 and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

5. Replace media every 2-3 days until cells reach 90% confluency. At first passage and subsequent passages, use Complete Thaw Medium 1.

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.25% Trypsin/EDTA.

2. Once the cells have detached, add Complete Thaw Medium 1 and transfer to a tube.

3. Spin down cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in Complete Thaw Medium 1.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:2 to 1:10 once or twice per week.

Cell Freezing

1. Aspirate the medium, wash the cells with PBS without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.25% Trypsin/EDTA.

2. Once the cells have detached, add Complete Thaw Medium 1 and count the cells.

3. Spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 4°C Cell Freezing Medium (BPS Bioscience #79796) at ~2 x 10^6 cells/ml.

4. Dispense 1 ml of cell suspension into each cryogenic vial. Place the vials in an insulated container for slow cooling and store at -80°C overnight.

5. Transfer the vials to liquid nitrogen the next day for long term storage.

Note: It is recommended to expand the cells and freeze at least 10 vials at an early passage for future use.

Validation Data

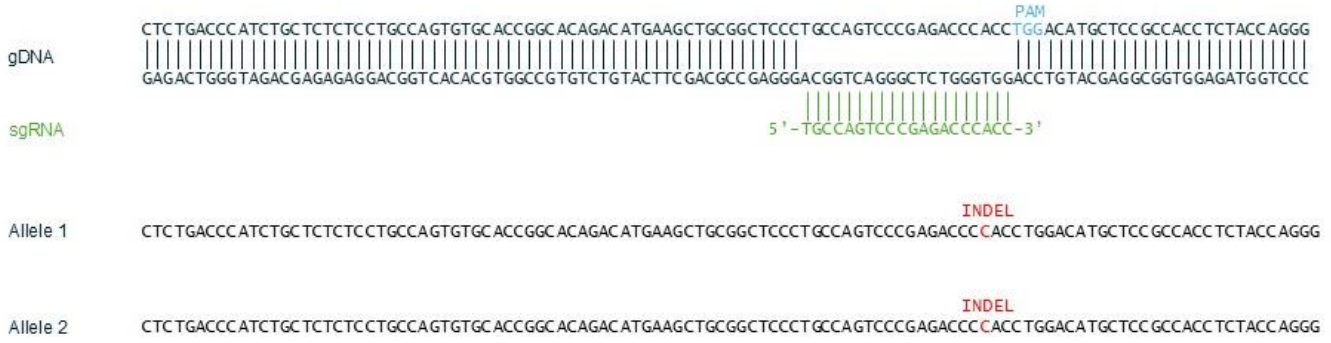

Figure 1: Genomic sequencing of HER2 in the HER2 Knockout MCF7 Cell Line.
Genomic DNA from the HER2 Knockout MCF7 cells was isolated and sequenced. The PAM (Protospacer Adjacent Motif) is shown in blue, the sgRNA (synthetic guide RNA) is shown in green, and the Indels (Insertions/Deletions) in the two HER2 alleles are highlighted in red. The HER2 genomic DNA is labeled as gDNA.

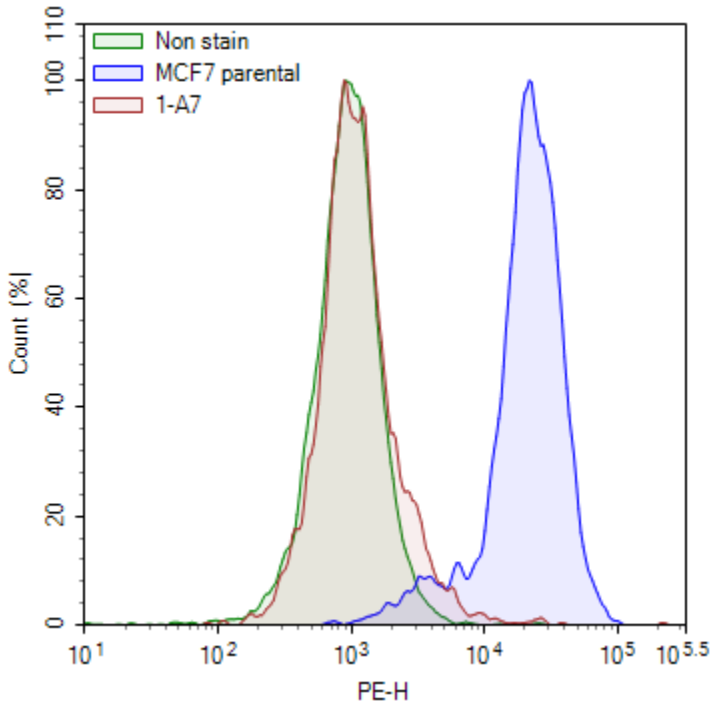

Figure 2. Expression of HER2 in the HER2 Knockout MCF7 Cell Line by flow cytometry.
HER2 Knockout MCF7 cells (red) or parental MCF7 cells (blue) were stained with PE anti-human CD340 (erbB2/HER-2) Antibody (BioLegend #324406) and analyzed by flow cytometry. Unstained parental MCF7 cells are shown in green. Y-axis is the % cell number. X-axis is the intensity of PE.

Data is representative.

License Disclosure
Visit bpsbioscience.com/license for the label license and other key information about this product.

Troubleshooting Guide
Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

Notes
The CRISPR/CAS9 technology is covered under numerous patents, including U.S. Patent Nos. 8,697,359 and 8,771,945, as well as corresponding foreign patents applications, and patent rights.

References
Schlam I. and Swain S., 2021 *npj Breast Cancer* 7: 56.

Related Products

Products	*Catalog #*	*Size*
HER2 (ERBB2) CHO Recombinant Cell Line (High, Medium, or Low Expression)	79612	2 vials
Chemi-Verse™ HER2 Kinase Assay Kit	82552	96 reactions
Afatinib	27009	50 mg
Androgen Luciferase Reporter 22RV1 Cell Line	78972	2 vials
Androgen Receptor Luciferase Reporter Lentivirus	78763	500 µl x 2
Estrogen Luciferase Reporter T47D Cell Line	82349	2 vials

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