Description

NY-ESO-1 Knockout A375 Cell Line is an A375 human melanoma cell line in which NY-ESO-1 (New York esophageal squamous cell carcinoma 1, also known as Cancer/Testis Antigen 1 or CTAG1B) is no longer expressed due to CRISPR/Cas9 genome editing. This cell line was generated by electroporation of A375 cells with ribonucleoprotein complexes of Cas9 and sgRNAs targeting *CTAG1B*.

This cell line has been validated by genomic sequencing, western blot, and in a co-culture assay. These cells do not activate T-cells expressing a TCR (T cell receptor) that specifically recognizes NY-ESO-1.

Background

NY-ESO-1 (New York esophageal squamous cell carcinoma 1, also known as Cancer/testis antigen 1, or CTAG1B), is an important tumorigenic marker present in malignant cells. Normally expressed only in embryonic testis, this highly immunogenic protein is not usually found in normal tissues, but can be re-expressed in cancers including melanoma, multiple myeloma (MM), non-small cell lung carcinoma (NSCLC), and breast and ovarian cancer, making it a promising candidate antigen for cancer immunotherapy. Several NY-ESO-1-directed therapies are being developed including cancer vaccines, anti-NY-ESO-1 adoptive cell therapy, and NY-ESO-1-specific TCR (T-cell receptor)-T cell therapy in combination with checkpoint inhibitors.

Application

- Useful as a negative control in experiments evaluating NY-ESO-1-directed therapies, such as TCR cells.

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

A375 cells, human melanoma cell line, epithelial cells, adherent.

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 6	BPS Bioscience #60183

Storage Conditions

Cells will arrive upon 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

Thaw Medium 6 (BPS Bioscience #60183):
DMEM medium supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Cell Culture Protocol

Note: A375 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 6.

   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 Thaw Medium 6.

3. Transfer the resuspended cells to a T25 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 Thaw Medium 6 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 Thaw Medium 6.

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 Thaw Medium 6 and transfer to a tube.

3. Spin down cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in Thaw Medium 6.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:2 to 1:10 once or twice a 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 Thaw Medium 6 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

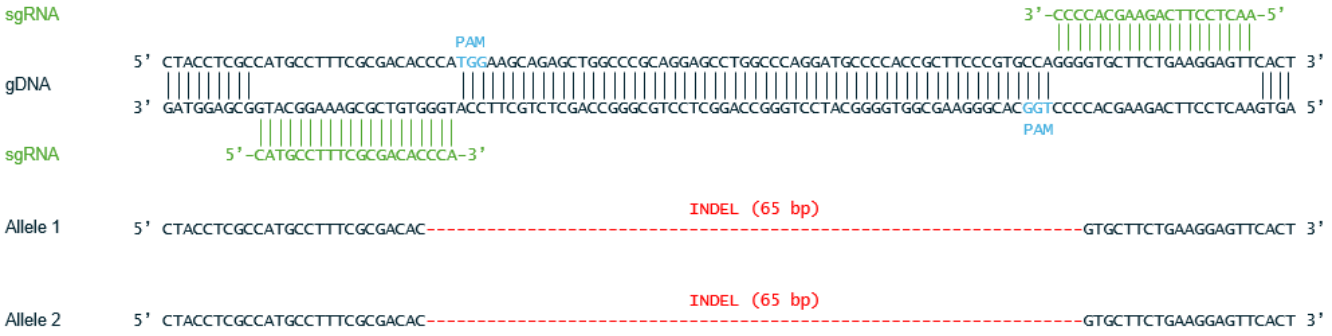

Figure 1. Genomic Sequencing of CTAG1B (NY-ESO-1) gene in the NY-ESO-1 Knockout A375 Cell Line.
Genomic DNA from the NY-ESO-1 Knockout A375 cells was isolated and sequenced. The PAMs (Protospacer Adjacent Motifs) are shown in blue, the sgRNAs (single guide RNAs) in green, and the Indel (Insertion/Deletion) in the NY-ESO-1 alleles are indicated in red.

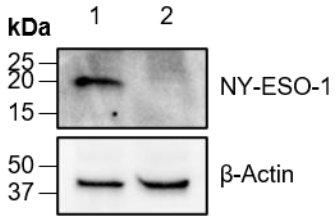

Figure 2. Expression of NY-ESO-1 in the NY-ESO-1 Knockout A375 cells assessed by Western Blot. Lysates from parental A375 cells (lane 1) and NY-ESO-1 Knockout A375 cells (lane 2) were run on a 4-20% SDS-PAGE gel and analyzed by Western blot with an anti-NY-ESO-1 monoclonal antibody, followed by an HRP-conjugated goat anti-mouse IgG antibody. As loading control, blots were stained with an anti-β-Actin monoclonal antibody, followed by an HRP-conjugated donkey anti-rabbit IgG antibody.

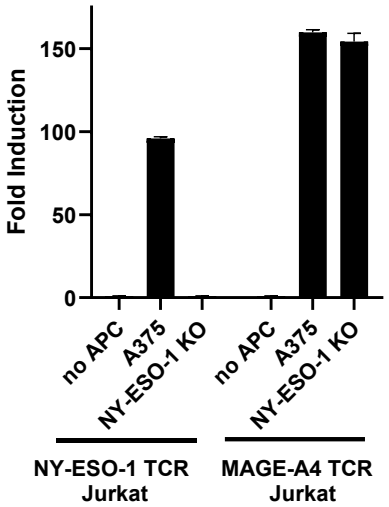

Figure 3. NY-ESO-1 Knockout A375 Cell Line co-culture assay with NY-ESO-1 TCR CD8+ NFAT Luciferase Reporter Jurkat Cell Line or MAGE-A4 TCR CD8+ NFAT Luciferase Reporter Jurkat Cell Line.

Parental A375 cells and NY-ESO-1 knockout (NY-ESO-1 KO) A375 cells were seeded at 20,000 cells/well in a 96-well plate overnight. Next day, cells were co-cultured with NY-ESO-1 TCR CD8+ NFAT Luciferase Reporter Jurkat cells (#78771) or MAGE-A4 TCR CD8+ NFAT Luciferase Reporter Jurkat cells (#78984) for 5 hours. Luciferase activity was measured. Results are shown as fold induction. A375 cells naturally present HLA-A*02:01 restricted NY-ESO-1 (amino acids 157-165) and MAGE-A4 (amino acids 230-239) peptide. Co-culture results in the activation of both NY-ESO-1 TCR CD8+ NFAT Luciferase Reporter Jurkat cells and MAGE-A4 TCR CD8+ NFAT Luciferase Reporter Jurkat cells. In contrast, NY-ESO-1 Knockout A375 do not activate NY-ESO-1 TCR CD8+ NFAT Luciferase Reporter Jurkat cells but maintain the ability to activate MAGE-A4 TCR CD8+ NFAT Luciferase Reporter Jurkat cells. Reporter Jurkat cells were not activated in the absence of antigen-presenting cells (no APC).

Data shown is representative.

License Disclosure

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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
Raza A., *et al.*, 2020 *J Transl Med* 18(1):140.
Thomas R., *et al.*, 2018 *Front Immunol* 9:00947.

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