

## Product Information

### A375 NY-ESO-1 Antigen Panel Cell Lines

Catalog Number **ATG004-1KT**

Store Temperature -196 °C (liquid nitrogen)

## TECHNICAL BULLETIN

### Product Description

The A375 NY-ESO-1 antigen panel is a series of three (3) genetically modified cell lines that targets the NY-ESO-1 locus in A375 lung carcinoma cells.

The panel consists of the following cell lines (Figure 1):

1. ATG004A-1VL: A375 NY-ESO-1 Knockout
2. ATG004B-1VL: A375 NY-ESO-1 Low
3. ATG004C-1VL: A375 NY-ESO-1 High

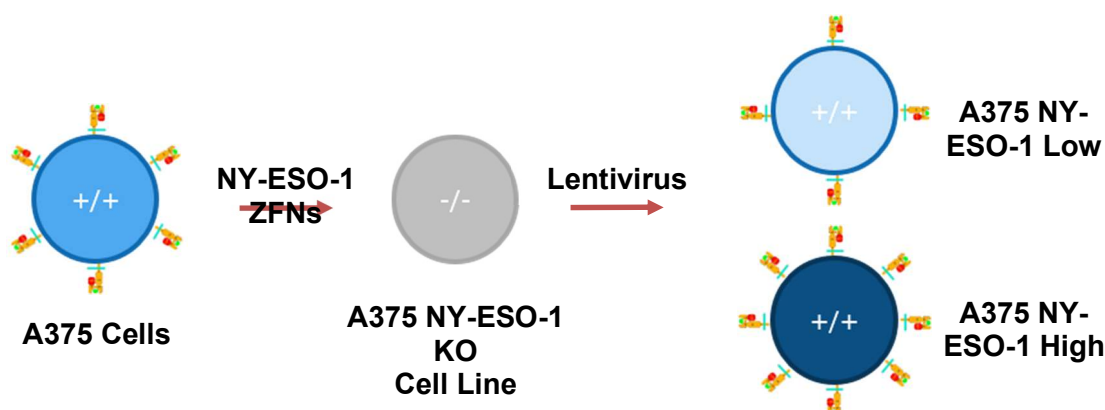
CompoZr® zinc finger nuclease (ZFN) technology was used to create a pan-allelic, targeted knockout (KO) of the NY-ESO-1 gene in wild type A375 cells.

Generation of the A375 NY-ESO-1 KO cell line was confirmed via next-generation sequencing (NGS) analysis as shown in Figure 2.

Following single cell cloning and expansion of the A375 NY-ESO-1 KO cell line, MISSION® lentiviral particles were used to randomly integrate and express exogenous, full-length NY-ESO-1 cDNA at low or high levels relative to the knockout to generate the A375 NY-ESO-1 Low (ATG004B-1VL) and A375 NY-ESO-1 High (ATG004C-1VL) cell lines.

NY-ESO-1 protein expression was measured in the antigen panel via RT-PCR as shown in Figure 4.

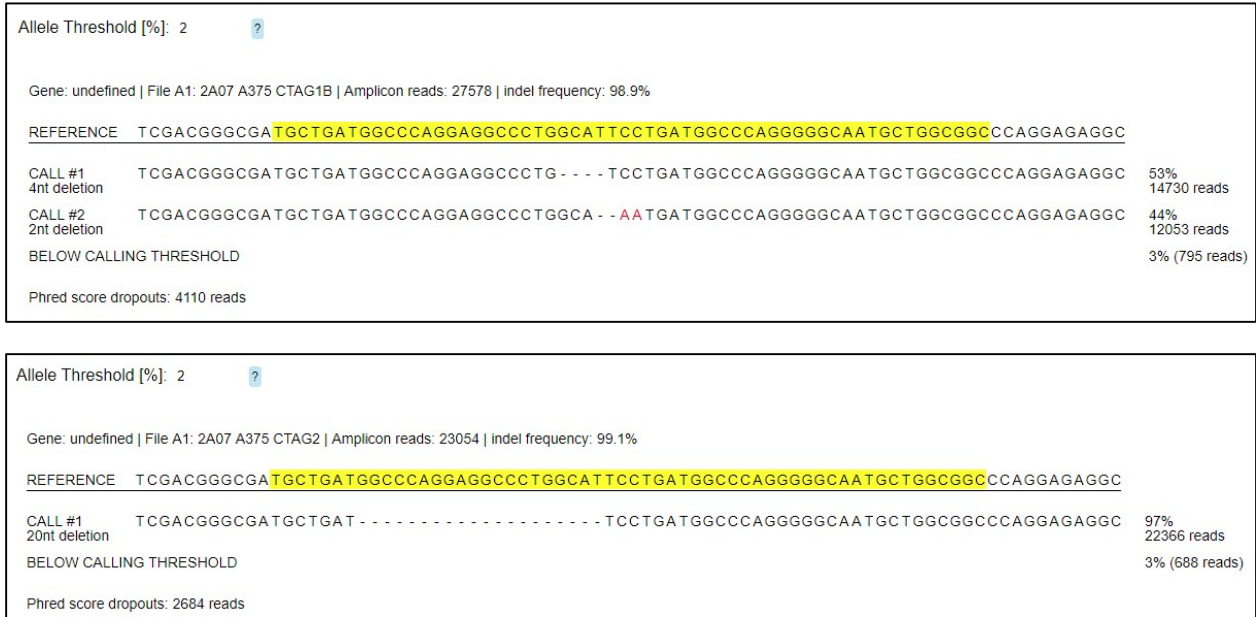
**Figure 1**  
**Generation of the Tumor-Associated Antigen Panel Cell Lines**



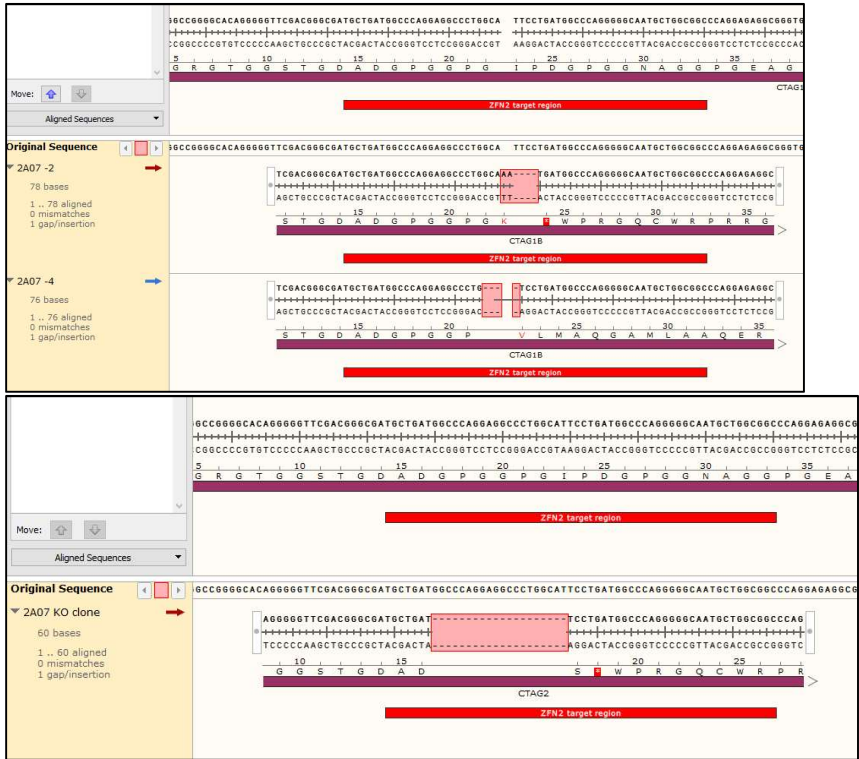
**Figure 2**  
**Genomic mutations in the NY-ESO-1 locus.**  
Site-specific pan-allelic -2/-4 bp and -20 bp insertions at the NY-ESO-1 locus in A375 cells

**Top panel:** Wild type A375 cells, ZFN target site is highlighted in yellow. -2/-4 bp deletions.

**Bottom panel:** Wild type A375 cells, ZFN target site is highlighted in yellow. -20 bp deletion.



**Figure 3.** Exonic alignment of -2/-4 deletions (top panel) and -20 deletion (bottom panel). All three deletions result in a predicted premature stop codon in exon 1 of human NY-ESO-1.



**Genomic sequence at the target region recognized by the ZFN pair.**

TGCTGATGGCCCAGGAGGCCCTGGCATTCTGATGGTCCAGGGGGCAATGCTGGCGGC

**NGS PCR for knockout**

Forward: TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNNNNNctgaaggagttcactgtgtccg

Reverse: GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGNNNNNNgcagaaagcactgcgtgatcca

**Figure 4****NY-ESO-1 Expression in the A375 NY-ESO-1 Antigen Panel via RT-PCR**

Expression of NY-ESO-1 was measured by RT-PCR using the primer pairs described above. Knock-out cells showed no detectable expression

|      | Ct probe set 1 | Ct gapdh |
|------|----------------|----------|
| KO   | No Ct          | 26.78    |
| KO   | No Ct          | 26.86    |
| Low  | 29.64          | 27.16    |
| Low  | 29.54          | 27.31    |
| High | 26.93          | 26.89    |
| High | 26.79          | 26.85    |

Expression levels of the high (red) and low (green) NY-ESO-1 knock-ins were measure by RT-PCR using the same probe set.

|      | Ct probe set 1 | Ct gapdh |
|------|----------------|----------|
| KO   | No Ct          | 26.78    |
| KO   | No Ct          | 26.86    |
| Low  | 29.64          | 27.16    |
| Low  | 29.54          | 27.31    |
| High | 26.93          | 26.89    |
| High | 26.79          | 26.85    |

Low clones express less NY-ESO-1 than high clones.

| 2 <sup>-ddCt</sup> | CTAG set 1 |
|--------------------|------------|
| Low                | 1          |
| High               | 5.13       |

## Components

This product is three (3) cryovials containing a minimum of 1 million A375 cells in each vial.

The cryoprotectant medium used is CryoStor® cell cryopreservation medium containing 10% DMSO (Catalog Number C2874).

### Cell Line Description

Organism: *Homo sapiens* (human)

Tissue: Carcinoma; Lung

Gender: Male

Morphology: Epithelial

Growth Properties: Adherent

### DNA Profile

STR-PCR Data:

Amelogenin: X

CSF1PO: 11,12

D13S317: 11,14

D16S539: 9

D18S51: 12,17

D21S11: 29,30

D3S1358: 15,17

D5S818: 12

D7S820: 9

D8S1179: 11,14

FGA: 23

Penta\_D: 9,15

Penta-E: 10,12

TH01: 8

TPOX: 8,10

vWA: 16,17

The STR profile of this cell line matches that of its parental cell line European Collection of Authenticated Cell Cultures (ECACC) Catalog Number 97011320. Please see the ECACC Catalog Number 97011320 datasheet for additional information about the origin of this cell line.

## Reagents and Equipment Required but Not Provided

- Dulbecco's Modified Eagle's Medium, high glucose, Catalog Number D5796
- Fetal Bovine Serum, USA origin, sterile-filtered, Catalog Number F2442
- Trypsin-EDTA solution, 1×, Catalog Number T4049
- Hank's Buffered Saline Solution (HBSS), Catalog Number H6648
- Biological safety cabinet

- 70% ethanol (prepared from Ethanol, Catalog Number E7148)
- Bio-Pure™ alcohol wipes, Catalog Number Z688487
- 37 °C water bath (operating range 35–38 °C)
- Sterile 15 mL conical tubes
- Centrifuge
- Serological pipettor with 1, 2, 5, 10, and 25 mL sterile pipettes
- Vacuum aspiration system and sterile plastic or glass aspiration tips
- Sterile 25 cm<sup>2</sup> or 75 cm<sup>2</sup> culture flasks
- 37 °C, 5% CO<sub>2</sub> incubator

## Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

**Precaution:** It is recommended that protective gloves and clothing always be used, and a full-face mask always be worn when handling frozen vials. It is important to note that some vials leak when submerged in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to the gas phase may result in the rapid expansion of the vessel, potentially blowing off its cap with dangerous force creating flying debris.

## Storage/Stability

Upon receiving a shipment of frozen cells, it is important the end user gives the shipment attention without delay. To ensure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70 °C. Storage at -70 °C will result in loss of viability.

At the time a cell line is ordered, end users should also consider the culture conditions for the new cell line and make sure the appropriate medium will be

## Procedures

### Medium Preparation Instructions

The base medium for this cell line is DMEM High Glucose Medium (Catalog Number D5796)

Complete Medium: To make the complete growth medium, add Fetal Bovine Serum (Catalog Number F2442) to a final concentration of 10%.

### Thawing of Frozen Cells

1. Thaw the vial by gentle agitation in a 37 °C water bath for ~1 minute. To reduce the possibility of contamination, keep the O-ring and cap out of the water.
2. Remove the vial from the water bath as soon as the contents are thawed and decontaminate by dipping in or spraying with 70% ethanol solution. All the operations from this point on should be carried out under aseptic conditions.
3. Transfer the cell suspension to a 15 ml conical tube containing 9 mL of warmed Complete Medium.
4. Centrifuge the cells at 125 × g for 5-7 minutes at room temperature.
5. Aspirate the media from the tube. Resuspend the cell pellet with 6 mL of warmed Complete Medium and plate into a 25 cm<sup>2</sup> or 75 cm<sup>2</sup> culture flask.
6. It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested, prior to the addition of the vial contents, the culture vessel containing the Complete Medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0-7.6) and temperature (37 °C).
7. Incubate the culture at 37 °C in a 5% CO<sub>2</sub> in air atmosphere incubator.

### Sub-culturing Procedure

Volumes used in the procedure are for a 75 cm<sup>2</sup> flask; proportionally reduce or increase volume of dissociation medium for culture vessels of other sizes.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with HBSS (Catalog Number H6648)
3. Add 2.0-3.0 mL of Trypsin-EDTA solution to flask and incubate at 37 °C for 6 minutes to detach the cells.
4. Add 6.0-8.0 mL of Complete Medium and aspirate cells by gentle pipetting.
5. Add appropriate aliquots of the cell suspension into new culture vessels. Sub-cultivation ratio: 1:4 to 1:20.
6. Incubate cultures at 37 °C in an incubator containing an atmosphere of 5% CO<sub>2</sub> in air.

### References

1. Giard, D.J., et al. *In vitro* cultivation of human tumors: establishment of cell lines derived from a series of solid tumors. *J. Natl. Cancer Inst.*, **51(5)**, 1417-23 (1973). PMID: 4357758

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Additional product and technical information can be obtained by searching for the catalog number at [sigmaaldrich.com](http://sigmaaldrich.com).

These products are covered by the Purchase Agreement as described in Exhibit 1.

## **Exhibit 1 – Purchase Agreement and Technology Label Licenses**

### **Tumor-Associated Antigen Panel Cell Lines**

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