

# Protocols for Cancer/Tumor cell lines

## Production

The Runtogen Cancer-related cell lines were generated in-house or licensed to Runtogen. All Cancer-related cell lines are supplied as a single vial containing  $>1 \times 10^5$  frozen cells in 1 mL of freezing medium. Please see the cell line-specific Certificate of Analysis (CoA) for the freezing medium composition and exact cell number. Each Cancer-related cell line is also supplied with the parental cell line as an additional control. The Runtogen Cancer-related cell lines are biological safety level 1 (BSL-1).

## Shipping and storage

The Runtogen Cancer-related cell lines are shipped on dry ice. While precautions have been taken to prevent CO<sub>2</sub> from entering the vial during shipment, it is suggested that upon receipt the cells be stored for two days or more in liquid nitrogen to allow any CO<sub>2</sub> to dissipate. When removing the vial from liquid nitrogen storage, leave at room temperature for approximately 30 seconds or longer to allow the liquid nitrogen to dissipate from the vial.

**Note: Always wear protective eyewear and gloves when handling vials stored in liquid nitrogen.**

## Quality Control

The Cancer-related cell lines have been tested for viability by post-bank thawing and cultivation, for sterility by Direct Inoculation of Tryptic Soy and Thioglycolate Broths, and verified to contain no Mycoplasma contamination (please see the cell line specific CoA for details). The Cancer-related cell lines are validated by PCR amplification and Sanger Sequencing to confirm the mutation at the genomic level. Primer sequences are on the cell line specific CoA.

## Starting cells from frozen cell stock (thaw quickly)

The following instructions are for resuspension in a T25 flask. A similar protocol with different resuspension volumes could be used to start cells in another appropriately sized vessel (i.e. T75 flask, 10 cm plate).

1. Remove the Cancer-related cell line from liquid nitrogen and place in a 37°C water bath for 2 minutes until nearly (~80%) thawed.
2. Remove the cells from the vial and add slowly into a 15 mL conical tube containing 9 mL pre-warmed Cell Culture Medium (see Appendix, Table 1).
3. Centrifuge for 3 minutes at  $300 \times g$  to pellet cells and remove the supernatant.
4. Add 2 mL of the appropriate Cell Culture Medium and transfer cells to a T25 flask containing 4 mL of pre-warmed Cell Culture Medium.

5. Place the cells in the 37°C incubator with 5% CO<sub>2</sub>.

6. Gently replace medium after 24 hours with 5-10 mL appropriate Cell Culture Medium and continue to culture at 37°C with 5% CO<sub>2</sub>.

## Sub-culturing cells

We recommend that you passage cells at least once before using them in your desired application. The cell culture guidelines and volumes below describes the sub-culturing protocol we use to passage cells in a standard T25 cell culture flask. Culture volumes for different flasks should be increased or decreased as per manufacturer's guidelines.

### Culturing adherent Cancer-related cell lines

**Cell lines:** A375, Cal12T, DLD-1, EBC-1, HACAT, HCT-116, hTERT-HME1, hTERT-IMECs, hTERT RPE, LIM1215, MCF10A, NCI-H838, NCI-H1975, Panc 04.03, RKO, SW48, VACO 432

Cell lines are typically passaged when 70-90% confluent. More specific details on each cell line can be found in Appendix, Table 1.

1. Carefully aspirate the growth medium from the cells. This is best done by tilting the flask or plate and removing the medium without touching the cell surface.
2. Gently wash cells with 5 mL PBS to remove the remaining medium.
3. Trypsinize the cells with appropriate volume of trypsin-EDTA Solution (GIBCO, Cat. #25300096). Place the flask in the 37°C incubator for ~ 2 minutes for cells to release from the flask.
4. Neutralize trypsin with equal volume of recommended Cell Culture Medium (see Appendix, Table 1) to resuspend the detached cells.
5. Pipette cells up and down ~ 5 times with a 10 mL pipette to obtain a single cell suspension, while avoiding frothing of medium.
6. Plate cells with recommended split ratio (Appendix, Table 1) into new sterile flasks or plates with appropriate Cell Culture Medium. Place the cells at 37°C with 5% CO<sub>2</sub>.

### Culturing Suspension Cancer-related cell lines

**Cell lines:** K-562, NALM-6

Cell lines are typically maintained at a cell density between  $1 \times 10^5$  and  $1 \times 10^6$  viable cells/mL.

1. Make sure the cells are evenly distributed in the medium, carefully take a small sample (e.g. 100µl) of the cells from the cell suspension and determine the total number of viable cells using a cell counter.

2. The volume of culture needed to achieve the desired seeding density can be calculated using the formula:

$$\frac{\text{Total volume in new flask (ml)} * \text{Desired seeding density (10e6)}}{\text{Cell count (10e6)}}$$

3. Seed the cell suspension into a new sterile flask or dilute the current culture with required volume of Cell Culture medium (see Appendix, Table 1). Place the cells at 37°C with 5% CO<sub>2</sub>.

**Note:** Cells may experience a lag period of approximately 1 week before stable growth is achieved. Each cell line may grow at different doubling times and Cancer-related cells may have a slightly different morphology to the parental.

## Culturing Loosely Adherent or Mixed Cancer-related cell lines

### Cell Line: KMS-11

1. Carefully aspirate the medium with the floating cells to an appropriately sized centrifuge tube.
2. Add appropriate volume of DPBS to cover the surface area of the flask, then gently agitate the flask to rinse the cells and collect the DPBS into the same centrifuge tube.
3. Trypsinize the cells with appropriate volume of trypsin-EDTA Solution (GIBCO, Cat. #25300096). Place the flask in the 37°C incubator for ~ 2 minutes for cells to release from the flask.
4. Neutralize trypsin with equal volume of recommended Cell Culture Medium (see Appendix, Table 1), then transfer the cell suspension to the centrifuge tube from step 1.
5. Centrifuge at 200g for 3 minutes to pellet the cells.
6. Pour off the supernatant then resuspend the pellet in appropriate volume of medium.
7. Plate cells with recommended split ratio (see Appendix, Table 1) into a new sterile flasks or plates with appropriate Cell Culture Medium. Place the cells at 37°C with 5% CO<sub>2</sub>.

## Freezing cells

### Freezing Adherent Cancer-related cell lines

1. Trypsinize and spin down cells at 300 x g for 3 minutes.
2. Count the cells using a cell counter and spin down the desired volume of cells for freezing at 300g for 3 minutes.
3. Resuspend cell pellet in freezing medium containing 45% RPMI basal medium (or basal medium specified on the cell line CoA) + 50% FBS + 5% DMSO.
4. Transfer cell suspension to suitable cryovials.
5. Transfer the vials to a suitable freezing container and store at -80°C.
6. The following day, transfer cells to liquid nitrogen.



**Technical Tips:** We strongly recommend freezing down and using cells at lower passage numbers.

## Freezing Suspension Cancer-related cell lines

1. Spin down the required volume of cells for freezing at 300g for 3 minutes after counting with a cell counter.
2. Resuspend the cell pellet in freezing medium containing 40% RPMI basal medium (or basal medium specified on the cell line CoA) + 50% FBS + 10% DMSO.
3. Transfer cell suspension to suitable cryovials.
4. Transfer the vials to a suitable freezing container and store at -80°C
5. The following day, transfer cells to liquid nitrogen.

## Freezing loosely adherent or mixed Cancer-related cell lines

1. Carefully aspirate the medium with the floating cells to an appropriately sized centrifuge tube.
2. Add appropriate volume of DPBS to cover the surface area of the flask, then gently agitate the flask to rinse the cells and collect the DPBS in the same centrifuge tube.
3. Trypsinize the cells with appropriate volume of trypsin-EDTA Solution (GIBCO, Cat. #25300096). Place the flask in the 37°C incubator for ~ 2 minutes for cells to release from the flask.
4. Neutralize trypsin with equal volume of recommended Cell Culture Medium (see Appendix, Table 1) then transfer the cell suspension to the centrifuge from step 1.
5. Centrifuge at 300g for 3 minutes to pellet the cells.
6. Resuspend the pellet in appropriate volume of medium, count the cell line using a cell counter and spin down the desired volume of cells for freezing at 300g for 3 minutes.
7. Resuspend cell pellet in freezing medium containing 45% RPMI basal medium (or basal medium specified on the cell line CoA) + 50% FBS + 5% DMSO.
8. Transfer cell suspension to suitable cryovials.
9. Transfer the vials to a suitable freezing container and store at -80°C.
10. The following day, transfer cells to liquid nitrogen.

## Frequently Asked Questions (FAQs)

### 1. What happens if I exceed the cell densities recommended?

Exceeding maximum recommended density will affect health and viability of the cells. If cell densities are exceeded, the best practice is to start with a fresh stock.

### 2. Are mutant cell lines provided with a wild type control?

Cell lines are provided as isogenic pairs - the modified mutant line and the originating wild type parental line.

### 3. If antibiotic resistance is specified on the CoA, does the Cancer-related cell line need to be maintained in selection?

The cells do not need to be maintained in selection as the mutation is permanent, and the cell line is clonal.

## Appendix

Table 1. Cell Culture Medium and Cell Line Maintenance tips

Cell name	Basal Medium	Supplements	Sub cultivation
A375	DMEM/F-12 (with 2.5 mM L-glutamine, 15 mM HEPES)	10% FBS 1% Pen/Strep	Split at 70-80% confluency, approximately 1:5-1:6
Cal12T	DMEM/F-12 (with 2.5 mM L-glutamine, 15 mM HEPES)	10% FBS 1% Pen/Strep	Split at 70-80% confluency, approximately 1:2-1:4
DLD-1	RPMI 1640 (with 2mM L-glutamine and 25mM sodium bicarbonate)	10% FBS 1% Pen/Strep	Split at 70-80% confluency, approximately 1:6-1:10
EBC-1	MEM (with 2mM L-glutamine and 25mM sodium bicarbonate)	10% FBS 1% Pen/Strep	Split at 70-80% confluency, approximately 1:4-1:6
HACAT	DMEM/F-12 (with 2.5 mM L-glutamine, 15 mM HEPES)	10% FBS 1% Pen/Strep	Split at 70-80% confluency, approximately 1:6-1:10
HCT116	RPMI 1640 (with 2mM L-glutamine and 25mM sodium bicarbonate)	10% FBS 1% Pen/Strep	Split at 70-80% confluency, approximately 1:10-1:20
hTERT-HME1	DMEM/F-12 (with 2.5 mM L-glutamine, 15 mM HEPES)	10% FBS 1% Pen/Strep 10 µg/mL insulin 20 ng/mL hEGF 0.5 µg/mL hydrocortisone	Split at 80-90% confluency, approximately 1:4-1:8
hTERT-IMECs	DMEM/F-12 (with 2.5 mM L-glutamine, 15 mM HEPES)	10% FBS 1% Pen/Strep 10 µg/mL insulin 20 ng/mL hEGF 0.5 µg/mL hydrocortisone	Split at 80-90% confluency, approximately 1:4-1:8
hTERT RPE-1	DMEM/F-12 (with 2.5 mM L-glutamine, 15 mM HEPES)	10% FBS 1% Pen/Strep	Split at 70-80% confluency, approximately 1:4-1:8
LIM1215	RPMI 1640 (with 2mM L-glutamine and 25mM sodium bicarbonate)	10% FBS 1% Pen/Strep 1 µg/mL insulin 1 µg/mL hydrocortisone	Split at 70-80% confluency, approximately 1:4-1:8. Medium change every 3-4 days.
MCF10A	DMEM/F-12 (with 2.5 mM L-glutamine, 15 mM HEPES)	5% horse serum 1% Pen/Strep 10 µg/mL insulin 20 ng/mL hEGF 0.5 µg/mL hydrocortisone 0.1 µg/mL cholera toxin	Split at 80-90% confluency, approximately 1:6-1:10
NCI-H838	RPMI 1640 (with 2mM L-glutamine and 25mM sodium bicarbonate)	10% FBS 1% Pen/Strep	Split at 70-80% confluency, approximately 1:12-1:20
NCI-H1975	RPMI 1640 (with 2mM L-glutamine and 25mM sodium bicarbonate)	10% FBS 1% Pen/Strep	Split at 70-80% confluency, approximately 1:10-1:12
Panc 04.03	RPMI 1640 (with 2mM L-glutamine and 25mM sodium bicarbonate)	10% FBS 1% Pen/Strep	Split at 70-80% confluency, approximately 1:2-1:6

## Appendix con...

Table 1. Cell Culture Medium and Cell Line Maintenance tips

Cell name	Basal Medium	Supplements	Sub cultivation
RKO	RPMI 1640 (with 2mM L-glutamine and 25mM sodium bicarbonate)	10% FBS 1% Pen/Strep	Split at 70-80% confluency, approximately 1:6-1:15
SW48	RPMI 1640 (with 2mM L-glutamine and 25mM sodium bicarbonate)	10% FBS 1% Pen/Strep	Split at 70-80% confluency, approximately 1:3-1:6
VACO 432	RPMI 1640 (with 2mM L-glutamine and 25mM sodium bicarbonate)	10% FBS 1% Pen/Strep	Split at 80-90% confluency, approximately 1:2-1:6
K-562	IMEM (with 2mM L-Glutamine and 25mM Sodium Bicarbonate)	10% FBS 1% Pen/Strep	Maintain at $1 \times 10^5 - 1 \times 10^6$ cells/ml
NALM-6	RPMI 1640 (with 2mM L-glutamine and 25mM sodium bicarbonate)	10% FBS 1% Pen/Strep	Maintain at $1.0-3.0 \times 10^6$ cells/ml, splitting approximately 1:6-1:10
KMS-11	RPMI 1640 (with 2mM L-glutamine and 25mM sodium bicarbonate)	10% FBS 1% Pen/Strep	Split at 70-80% confluency, approximately 1:4-1:6

### Abbreviation and catalog numbers:

DMEM/F-12: Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (Thermo Fisher, Cat. #31330-038)

FBS: Fetal Bovine Serum (Thermo Fisher, Cat. #10270-106)

IMDM: Iscove's Modified Dulbecco's Medium (Thermo Fisher, Cat. #21980-032)

MEM: Minimum Essential Medium (Thermo Fisher, Cat. #31095-029)

Pen/Strep: Penicillin/Streptomycin

RPMI1640: Roswell Park Memorial Institute Medium (Thermo Fisher, Cat. #21875-034)

### For more information

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