TrypKit™ Subculture Reagent Kit
Specification and Instruction Sheet

Safety and Use Statement
TrypKit™ is for research use only. This product is not approved for human or veterinary use or for use in *in vitro* diagnostics or clinical procedures.

Component Storage
Phosphate Buffered Saline (PBS, CM-0001) should be stored at room temperature. 0.05% Trypsin/0.02% EDTA Solution (CM-0017, and CM-0019) and Trypsin Neutralizing Solution (CM-0008 and CM-0018) may be stored at -20°C until their expiration date or at 4-8°C for up to 1 month.

Product	Part No.	Volume	Storage	Volumes to use		
Small TrypKit™	**LL-1010**			Per 1 cm^2	Per T25 Flask	Per T75 Flask
Phosphate Buffered Saline (PBS) without calcium or magnesium	CM-0001	500 mL	RT	0.2 mL	5 mL	15 mL
0.05% Trypsin/0.02% EDTA (phenol red-free)	CM-0019	25 mL	-20°C	0.02 mL	0.5 mL	1.5 mL
Trypsin Neutralizing Solution (TNS)	CM-0008	25 mL	-20°C	0.02 mL	0.5 mL	1.5 mL
Large TrypKit™	**LL-0013**					
Phosphate Buffered Saline (PBS) without calcium or magnesium	CM-0001	500 mL	RT	0.2 mL	5 mL	15 mL
0.05% Trypsin/0.02% EDTA (phenol red-free)	CM-0017	100 mL	-20°C	0.02 mL	0.5 mL	1.5 mL
Trypsin Neutralizing Solution (TNS)	CM-0018	100 mL	-20°C	0.02 mL	0.5 mL	1.5 mL

Product	Quality Control Testing			
Phosphate Buffered Saline (PBS)	USP Sterility	pH 7.4 ±0.2	Osmolality 280 ±20 mOsm	Cell Passaging Evaluation
0.05% Trypsin/0.02% EDTA	USP Sterility	pH 7.6 ±0.4	Osmolality 290 ±20 mOsm	Cell Passaging Evaluation
Trypsin Neutralizing Solution (TNS)	USP Sterility	pH 7.5 ±0.3	Osmolality 290 ±20 mOsm	Cell Passaging Evaluation

The Lifeline Guarantee
Lifeline's rigorous quality control ensures sterility and performance to standardized testing criteria. If Lifeline's products do not meet your expectations or our posted performance and quality standards, we will replace them at no charge or provide a full refund. Upon request, Lifeline will provide lot-specific QC test results, material safety data sheets and certificates of analysis. See complete guarantee/warranty statement at lifelinecelltech.com or contact your Lifeline representative for more information.

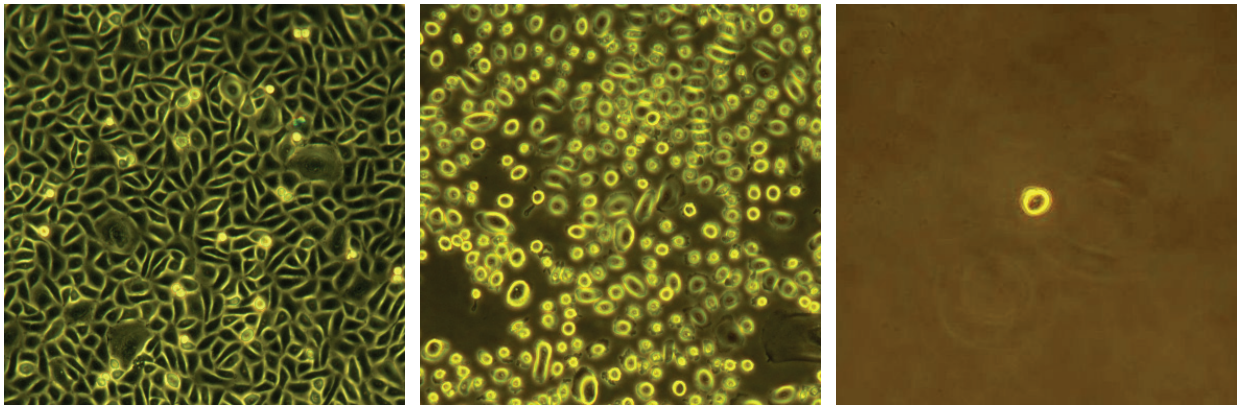
Basic Sterile Technique

TrypKit™ should only be used in a sterile environment, a Class II biological safety cabinet with front access and filtered laminar airflow, or an equivalent device. Always wear gloves and eye protection when working with these materials. Wipe or spray medium bottle and LifeFactors with 70% ethanol or isopropanol before opening, especially around the area of the cap. Make sure these surfaces have dried before opening the bottle. Transfer of medium or LifeFactors should be done with disposable sterile pipettes. Do not mouth pipette! Take up the volume needed into the pipette, being careful not to touch the sterile tip to the rim of the container or any other surface. Close the container and open the container into which the transfer is being made, again being careful not to touch any surfaces with the sterile tip. Transfer the material and close the container. Wash your hands before and after working with cell cultures. Do not block airflow in a laminar flow hood as this may compromise sterility. Be sure hood air filters are replaced regularly and the biological cabinet is certified routinely.

Quick Steps for Passaging Cells

1. All steps must be completed under sterile conditions in a biological safety cabinet.
2. If the cell culture medium contains serum, each flask should be rinsed with Lifeline's Phosphate Buffered Saline (PBS, CM-0001) twice prior to adding the Lifeline's 0.05% Trypsin/0.02% EDTA (CM-0017 or CM-0019).
3. Add the appropriate volume of 0.05% Trypsin/0.02% EDTA to each flask, see table on page 1.
    a. Some strongly adherent cell types, such as keratinocytes, may require double the recommended volume.
4. Incubate the cells at room temperature or 37°C with the trypsin for 1-3 minutes.
    a. Some strongly adherent cell types, such as keratinocytes, may take much longer and may require trypsinization at 37°C.
    b. Over-trypsinization may damage cells.
5. Once the cells have started to detach (observe with a microscope), gently tap the flask(s) to completely detach the cells.
    a. Some cell types may require more vigorous tapping.
6. Add an equal volume of Lifeline's TNS (CM-0008 or CM-0018) to each flask and mix to inactivate the trypsin and EDTA.
7. Using safe laboratory techniques pipette the cells into a sterile centrifuge tube.
8. Add Lifeline's PBS (CM-0001) to the culture vessel to ensure all the cells are collected and pipette this into the sterile centrifuge tube along with the other cells.
9. Check culture vessel under the microscope for cells still attached and repeat steps if necessary to retrieve all the cells from the vessel.
10. Centrifuge cells at 150 x g for 3-5 minutes.
    a. For best results, calculate speed for individual centrifuge type.
    b. Time may also be centrifuge dependent.
    c. Do not over centrifuge cells as this may cause cell damage.
    d. After centrifugation, the cells should form a clean loose pellet.
11. Please consult Lifeline's technical service if issues arise from trypsinization or centrifugation.
12. Carefully aspirate neutralized trypsin from the centrifuge tube.
13. Re-suspend the cell pellet in pre-warmed LifeLine brand culture medium by gently pipetting up and down with a 2 or 5 mL pipette.

LifeLine keratinocytes before, during and after trypsinization, 100X magnification.

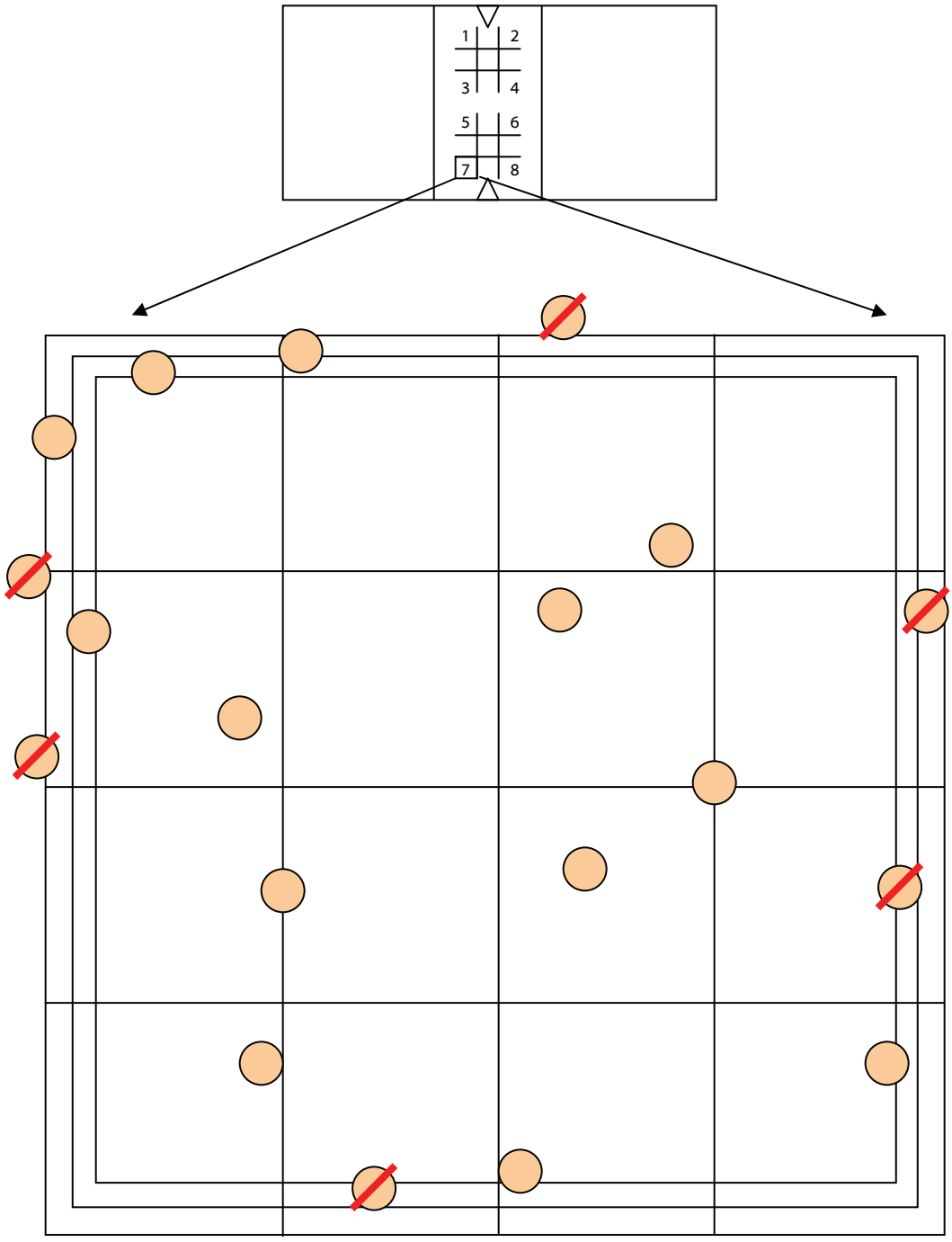


Cell Counting procedure

1. Resuspend cells in the appropriate volume of medium (defined in the procedure being used).
2. Transfer 20 µL of cell suspension into a microfuge tube.
3. Dilute the cells in one or more of the following ways:
   a) Dilution factor of 2: add 20 µL of Trypan Blue and mix.
   b) Dilution factor of 5: add 80 µL Trypan Blue and mix.
   c) Dilution factor of 10: add 180 µL Trypan Blue and mix.
4. Allow at least one minute but no longer than fifteen minutes exposure to Trypan blue before counting cells for a reliable viability estimate.
5. Load 10 µL of cell suspension into each chamber of a clean hemacytometer.
6. Count the cells in 8 quadrants (see attached diagram on the next page) using the following rules:
   a) Count all the cells within the triple-line border of each quadrant.
   b) If a cell is touching the center line of the triple-line border on the top or left side, it is counted.
   c) If a cell is touching the center line of the triple-line border on the right or bottom, it is **NOT** counted.
   d) If you count fewer than 15 cells in one quadrant or greater than 65 cells in one quadrant it is important to concentrate or dilute the cells.
   e) If you count fewer than 120 total cells, centrifuge the cell suspension and resuspend it in a lower volume and re-count.
   f) If you count greater than 520 cells, prepare a new dilution.
7. Calculate the concentration of viable cells:

$$\frac{\text{Total viable cells}}{\text{8 (quadrants)}} \times 10{,}000 \times \text{dilution factor}$$

Note: 10,000 is the factor to convert the volume of one quadrant (0.1 µL) into 1 mL.



Visual example of counting cells in 1 of the 8 quadrants using a hemacytometer. There are 13 cells within the counted region of this quadrant and 6 that would not be counted.