Specification and Instruction Manual

# TrypKit™ and TrypKit Xeno-Free§ Subculture Reagent Kits

LL-0013

This product 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.


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## Safety and Use Statement

TrypKit and TrypKit Xeno-Free (XF) are for Research Use Only. These products are not approved for human or veterinary use or for use in *in vitro* diagnostics or clinical procedures.

Trypsin Neutralizing Solution Xeno-Free contains human serum and should be handled as potentially infectious material. While the human serum used in these products have been tested, no known test method can guarantee the absence of Hepatitis B virus, Hepatitis C virus, HIV-1, HIV-2 or other infectious agents. All products which contain or have contacted human blood based products should be handled as Biosafety Level 2 (www.CDC.gov) and according to the Bloodborne Pathogen Standard 29 CFR 1910.1030.

## Component Storage

Phosphate Buffered Saline (PBS, CM-0001) should be stored at room temperature. The 0.05% Trypsin/0.02% EDTA Solutions (CM-0017 and CM-0046) and Trypsin Neutralizing Solutions (CM-0018 and CM-0047) may be stored at -20°C until their expiration date or at 2 to 8°C for up to 1 month.

| Product | Part No. | Volume | Storage | Volumes to Use | | |
|---|---|---|---|---|---|---|
| | | | | Per 1 $cm^2$ | Per T25 Flask | Per T75 Flask |
| **TrypKit** | LL-0013 | Kit | | | | |
| 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 | 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 |
| **TrypKit Xeno-Free** | LL-0043 | Kit | | | | |
| Phosphate Buffered Saline (PBS) without calcium or magnesium | CM-0001 | 500 mL | RT | 0.2 mL | 5 mL | 15 mL |
| Trypsin/EDTA XF | CM-0046 | 100 mL | -20°C | 0.02 mL | 0.5 mL | 1.5 mL |
| Trypsin Neutralizing Solution XF (TNS-XF) | CM-0047 | 100 mL | -20°C | 0.02 mL | 0.5 mL | 1.5 mL |

| Product | Quality Control Testing | | | |
|---|---|---|---|---|
| | Sterility | pH | Osmolality | Cell Passaging |
| PBS without calcium or magnesium | Sterile | 7.4 ± 0.2 | 280 ± 20 mOsm | Confirmed |
| 0.05% Trypsin/0.02% EDTA and Trypsin/EDTA XF | Sterile | 7.6 ± 0.4 | 290 ± 20 mOsm | Confirmed |
| TNS and TNS-XF | Sterile | 7.5 ± 0.3 | 290 ± 20 mOsm | Confirmed |

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*[§]Lifeline documents that all materials used in the manufacture of products which are labeled 'Xeno-Free' have never come into contact with material of animal (non-human) origin and are not of animal (non-human) origin. Trypsin Neutralizing Solution Xeno-Free contains human serum.*

## Basic Aseptic Technique

Reagents, cells, medium, and LifeFactors™ should only be used in an aseptic 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 all bottles and vials with 70% ethanol or isopropanol, especially around the area of the cap, before placing them in the biological safety cabinet. Allow these surfaces to dry completely before opening the bottle or vials. Transfer materials 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. Ensure that biological safety cabinets are certified routinely and that the HEPA filters are replaced regularly.

## Quick Steps for Passaging Cells

*When culturing cells in a Xeno-Free[§] environment, only use media and reagents that are documented to be Xeno-Free so as to maintain the Xeno-Free status of certain cell cultures.*

1. **Always wash hands before and after working with cell cultures.**
2. **Always wear eye protection and gloves when working with cell cultures.**
3. **Always use a certified biological safety cabinet when working with cells, medium, or reagents.**
4. **All steps must be completed under sterile conditions in a biological safety cabinet.**
5. If the cell culture medium contains serum, each flask should be rinsed with Lifeline® PBS twice prior to adding Lifeline 0.05% Trypsin/0.02% EDTA or Trypsin/EDTA XF.
6. Add the appropriate volume of Trypsin/EDTA to each flask, see table on page 1.

   *Note: Some strongly adherent cell types, such as keratinocytes, may require double the recommended volume.*
7. Incubate the cells at room temperature or 37°C with the trypsin for 1 to 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.
8. Once the cells have started to detach (observe with a microscope), gently tap the flask(s) to completely detach the cells.

   *Note: Some cell types may require more vigorous tapping.*
9. Add an equal volume of Lifeline's TNS or TNS-XF to each flask and mix to inactivate the Trypsin/EDTA.
10. Using aseptic laboratory techniques pipette the cells into a sterile centrifuge tube.

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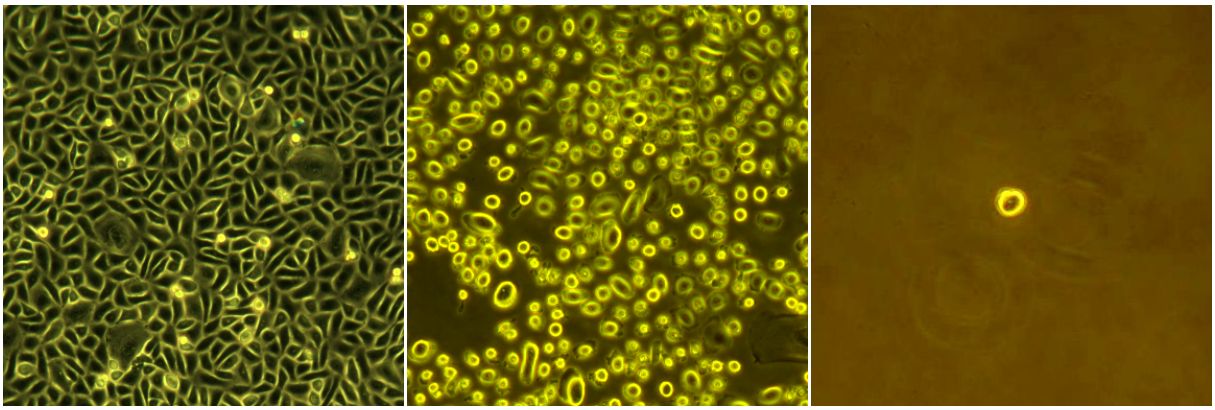
11. Add Lifeline PBS to the culture vessel to ensure all the cells are collected and pipette this into the sterile centrifuge tube along with the other cells.
12. Check culture vessel under the microscope for cells still attached and repeat steps if necessary to retrieve all the cells from the vessel.
13. Centrifuge cells at 150 x *g** for 3 to 5 minutes. (See page 4 for RCF equation.)
    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.
14. Carefully aspirate neutralized trypsin from the centrifuge tube.
15. Re-suspend the cell pellet in the respective pre-warmed Lifeline culture medium by gently pipetting up and down with a 2 or 5 mL pipette.
16. Add the respective pre-warmed Lifeline culture medium to new flasks (1 mL per 25 $cm^2$).
17. Inoculate cells as per the respective Cell Instruction Sheet.

***To calculate RCF (x *g*):** **$RCF = 0.00001118 \times (rpm)^2 \times r$**
r = rotational radius in centimeters
rpm = rotations or revolutions per minute



*From right to left, Lifeline® keratinocytes before, during and after trypsinization (100X).*

## Cell Counting Procedure

1. Resuspend cells in a volume of the appropriate medium (see respective Cell Instruction Sheet).
2. Transfer 20 µL of cell suspension into a microcentrifuge tube.
3. Dilute the cells in the microcentrifuge tube to one of the following Dilution Factors:
    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 the Cell Suspension/Trypan Blue mixture to sit for up to 1 to 5 minutes 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 diagram on page 4) 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, respectively.
   e. If you count fewer than 120 total cells, concentrate the cells and re-count.
   f. If you count greater than 520 cells, prepare a new dilution.
7. Calculate the concentration of viable cells:

$$\frac{\text{Total viable cells}}{8\ \text{(quadrants)}} \times 10{,}000 \times \text{dilution factor} = \#\ \text{of viable cells per mL}$$

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

## The Lifeline Guarantee

Lifeline's rigorous quality control ensures sterility and performance to standardized testing criteria. Upon request, we 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 technical representative for more information.

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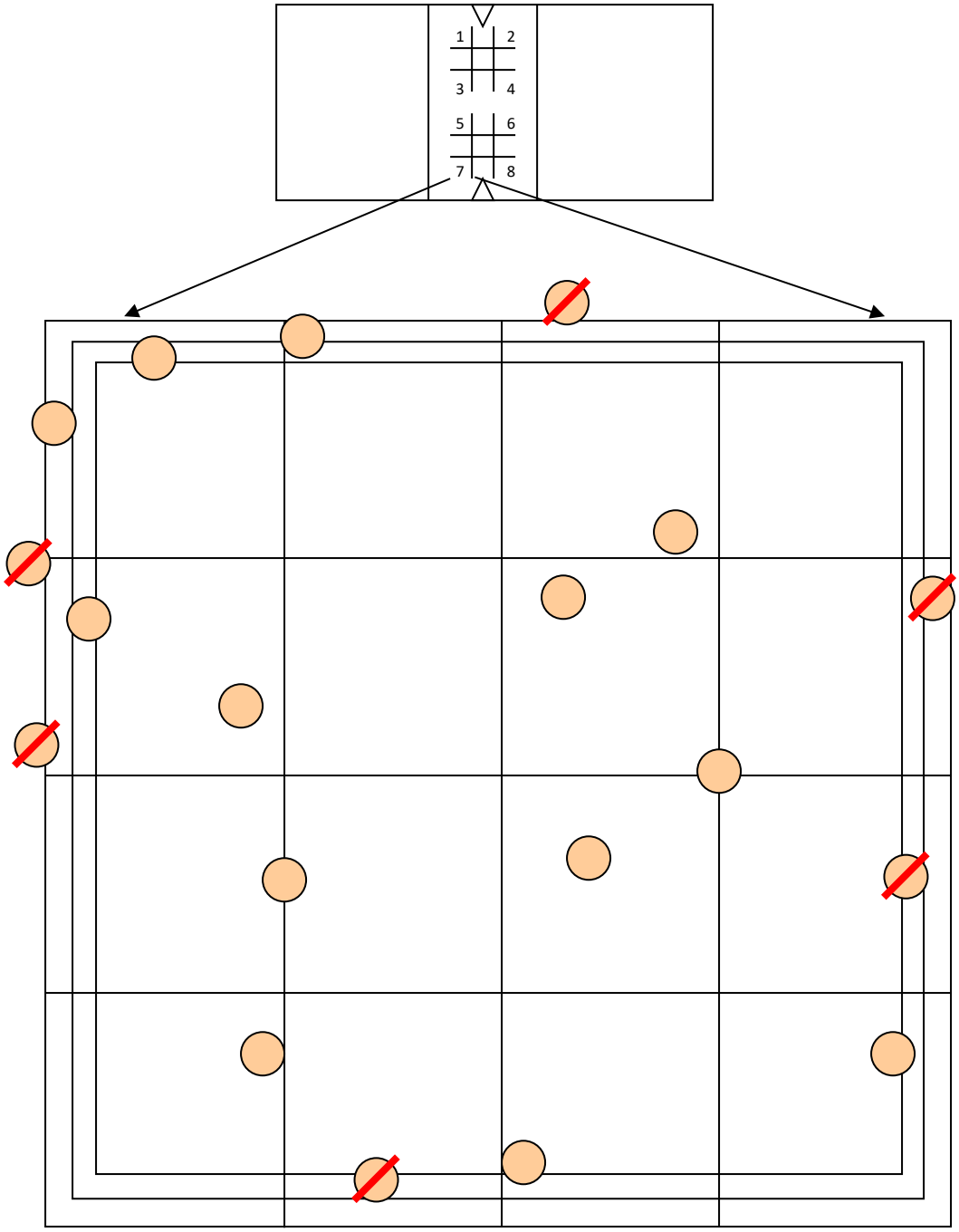


*Visual example of counting cells in 1 of the 8 quadrants using a hemacytometer. There are 13 cells within the counted region of this quadrant and 6 that would not be counted.*