EZ-LiFT™ Stem Cell Passaging Reagent

Dissociation Reagent

Cat. # SCM139-100ML

pack size: 100mL

Store at Room Temp

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.
NOT FOR HUMAN OR ANIMAL CONSUMPTION.

Data Sheet

Background

A common problem associated with the routine culture of human pluripotent stem cells, including ES and iPS cells, is the unwanted spontaneous differentiation of cells. Without manual intervention, differentiated cells proliferate quickly and eventually diminish the overall quality of the pluripotent stem cell culture. Manual removal of differentiated cells is laborious and time consuming, making it difficult to process multiple hES/iPS cell lines simultaneously. Moreover, the risk for contamination is present each time the culture is subjected to this clean-up process.

EZ-LiFT™ Stem Cell Passaging Reagent is a proprietary enzyme-free and chemically defined stem cell dissociation reagent that selectively passages only undifferentiated pluripotent stem cells. The reagent eliminates the need for manual removal of differentiated cells and produces high cell viability without the need for ROCKi. By nature of its selectivity for undifferentiated pluripotent stem cells, EZ-LiFT can be used to rescue highly differentiated hES/iPS cultures, thus enabling the recovery of precious samples and time.

EZ-LiFT does not suffer from the drawbacks of other enzyme-based dissociation reagents such as Dispase, Collagenase IV and Accutase. The disadvantages of enzyme-based reagents are that they still require manual removal of differentiated cells, suffer from lot-to-lot variability and may carry the risk for karyotypic abnormalities.

Features and Benefits

- Enzyme-free and chemically defined formulation: Produces consistent results.
- Enriches for pluripotent stem cell populations: No manual clean-up required.
- Rescues highly differentiated hES/iPS culture: Saves precious samples and time.
- Gentle on cells with high cell survival rates: ROCKi is optional, but not required.

Storage

EZ-LiFT Stem Cell Passaging Reagent can be stored at room temperature for 1 year without loss of activity.

Quality Control

pH: 7.2-7.4

Sterility Tested: Pass

Osmolarity: 340-370 mOsm

Rev.1.0/2018-03-15/SCM139-100ML/NJA

Protocols:

Important Notes: Parameters important to maintain high quality pluripotent cultures are outlined below.

- **Incubation time**: Colony dissociation is time dependent. To ensure high quality, passage **no more than** 3 wells at a time. The protocols outlined below are based on passaging one well of a 6-well plate.
- **Colony confluence:** The optimal cell confluence is 60-80% confluency. However, overconfluent cultures can still be rescued by longer incubation time with EZ-LiFT Reagent (see protocol below).
- **Temperature:** For the most consistent results, we recommend that the EZ-LiFT Reagent and the shaker (if used) be warmed to 37°C before starting. Minimize the time that the cells are not at 37°C. Do not rinse your cell culture with any ice-cold solution or medium before treatment with EZ-LiFT as the cold temperature may slow down the rate of colony dissociation. If cells are washed with ice cold medium, re-incubate culture at 37°C for at least 30 minutes before applying EZ-LiFT Reagent.
- **Shaking speed**: The protocol was optimized using a Labnet VorTemp 56 orbital shaker with 500 rpm as the optimal speed. If using another shaker, optimal speed may need to be determined.
- **Do not over-pipette.** After treatment with EZ-LiFT, detached clumps are fragile and can easily break apart into single cells.

EZ-LiFT Passaging Without a Shaker:
The protocol is based on 1 well of a 6-well plate. Do not passage more than 3 wells at a time.

1. Start with high-quality human ES or iPS culture that is 60-80% confluent. Warm EZ-LiFT Reagent to 37°C before starting.
   Critical Note: *Do not use ice-cold EZ-LiFT Reagent as this will slow down the colony dissociation.*
2. Aspirate the culture medium and wash wells twice with 1.5 mL EZ-LiFT Reagent. Aspirate after each wash.
3. Add 1 mL of EZ-LiFT reagent to each well. Incubate the plate at 37°C for 4 minutes.
4. After 4 minutes, tap rapidly on the bottom of the plate (i.e. 20-25 taps in 5 secs).
5. Place the plate back in the 37°C incubator for an additional 4 minutes.
6. After 4 minutes, tap rapidly on the bottom of the plate (i.e. 20-25 taps in 5 secs).
   Important*: Do not rinse the wells.*
7. Perform a quick microscopic inspection of the well(s).
   a. If a significant number of detached clumps are visible (Figure 2A-C), proceed to step 8.
   b. If no obvious detachment is observed (Figure 1C), repeat steps 4-7 except that in step 5, the 37°C incubation should be for 2 instead of 4 minutes. Proceed to step 8.
8. Gently collect the cell suspension (~1 mL) and transfer to a 15 mL conical tube. Neutralize with 5 mL of culture medium by gently adding the medium to the cell suspension. ***Do not pipette up and down as this may break cell clumps into single cells suspension*****.**
9. Centrifuge at 800 rpm or 130 x g for 3 minutes. Aspirate the supernatant.
10. Gently resuspend the cell pellet in 1 mL pluripotent medium (i.e. mTeSR or PluriSTEM).
    Caution: Do not pipette up and down more than two times. Over-pipetting may result in single cell dissociation.
11. Passage dissociated cell clumps to newly coated 6 well plates. If you are a first time user, we recommend passaging at a conservative 1:5 split ratio. After becoming familiar with the protocol, the split ratio may be increased to 1:10 up to 1:30 split ratio. Monitor cells daily. Newly passaged ES/iPS cells will typically reach 60-80% confluence in 6-8 days depending upon the split ratio.

EZ-LiFT Passaging Using a Shaker:
Important Note: The protocol was developed using a Labnet VorTemp™ 56 orbital shaker (National Labnet Co. Inc., Woodbridge, NJ) with 3 mm shaking orbit. The protocol is based on 1 well of a 6-well plate. Do not passage more than 3 wells at a time.

1. Start with high-quality human ES or iPS culture that is 60-80% confluent. Warm EZ-LiFT Reagent to 37°C before starting.
   Critical Note: *Do not use ice-cold EZ-LiFT Reagent as this will slow down the colony dissociation.*
2. Place a Labnet VorTemp™ 56 orbital shaker into the 37°C incubator. Be sure to wipe down the shaker with 70% ethanol first.
3. Aspirate the culture medium and wash wells twice with 1.5 mL EZ-LiFT Reagent. Aspirate after each wash.
4. Add 1 mL of EZ-LiFT reagent to each well.
5. Seal the plate with paraffin to prevent cross-contamination while shaking.
6. Shake plate at 500 rpm for 5 minutes at 37°C.
7. After 5 minutes, tap rapidly on the bottom of the plate (i.e. 20-25 taps in 5 secs).
   Important*: Do not rinse the wells.*
8. Perform a quick microscopic inspection of each well.
   a. If a significant number of detached clumps are visible (Figure 2A-C), proceed to step 9.
   b. If no obvious detachment is observed (Figure 1C):
      1. Tap rapidly on the bottom of the plate (i.e. 20-25 taps in 5 secs).
      2. Shake for 1 minute
      3. Tap rapidly on the bottom of the plate (i.e. 20-25 taps in 5 secs). Procced to Step 9.

9. Gently collect the cell suspension (~1 mL) and transfer to a 15 mL conical tube. Neutralize with 5 mL of culture medium by gently adding the medium to the cell suspension. ***Do not pipette up and down as this may break cell clumps into single cells suspension.***
10. Centrifuge at 800 rpm or 130 x g for 3 minutes. Aspirate the supernatant.
11. Gently resuspend the cell pellet in 1 mL pluripotent medium (i.e. mTeSR or PluriSTEM).
    Caution: Do not pipette up and down more than two times. Over-pipetting may result in single cell dissociation.
12. Passage dissociated cell clumps to newly coated 6 well plates. If you are a first time user, we recommend passaging at a conservative 1:5 split ratio. After becoming familiar with the protocol, the split ratio may be increased to 1:9 up to 1:30 split ratio. Monitor cells daily. Newly passaged ES/iPS cells will typically reach 60-80% confluence in 6-8 days depending upon the split ratio.

Cell Cryopreservation:

1. Start with high-quality human ES or iPS culture that is 60-80% confluent. Warm EZ-LiFT Reagent to 37°C before starting.
2. Treat wells with EZ-LiFT as outlined in the protocols above. However instead of passaging to newly coated 6-well plates, resuspend cell pellet in 1 mL pluripotent media (i.e. mTeSR1 or PluriSTEM) containing 10% DMSO.
   Caution: *Do not pipette up and down more than two times. Over-pipetting may result in single cell dissociation*.
3. One well of EZ-LiFT treated cells may be frozen into 3-5 cryovials. Depending upon the desired number of cryovials, add the requisite volume of pluripotent media containing 10% DMSO. For example, to obtain 5 cryovials, add 4 mL pluripotent media containing 10% DMSO to the 1 mL cell suspension in step 2.
4. Transfer cryovials to Nalgene slow freeze Mr. Frosty container and store at -80°C.
5. After 24 hours, transfer cryovials to liquid nitrogen for long-term storage.

Timecourse of EZ-LiFT passaging:

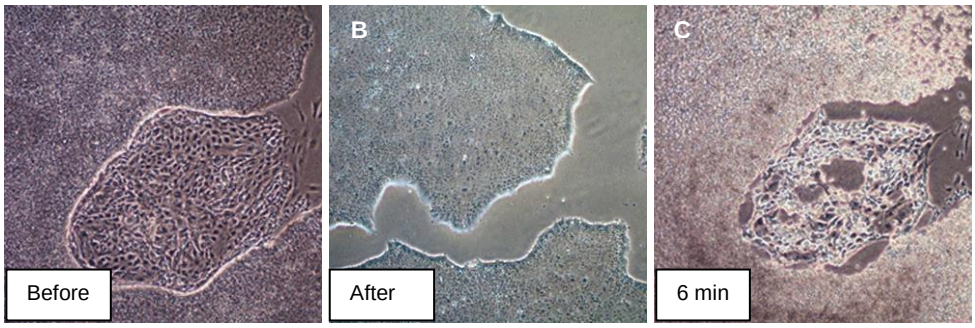

Figure 1. Human iPS cells before passaging with EZ-LiFT contain areas of differentiation (**A**). Six to eight days after passaging with EZ-LiFT™, human iPS colonies are free of differentiated areas (**B**). Incubation of human iPS colony in Figure 1A with EZ-LiFT was monitored for 18 minutes to illustrate the effects of EZ-LiFT on colony detachment. At 6 minutes incubation with EZ-LiFT, very little colony detachment was observed (**C**).

Optimal EZ-LiFT incubation times: Significant cell clumps are detached.

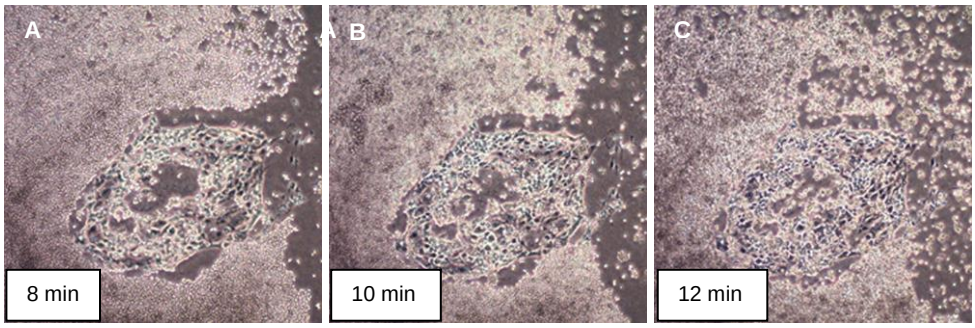

Figure 2. At 8-12 minutes incubation with EZ-LiFT, significant amounts of detached clumps are observed (**A-C**). 8-12 minutes are thus the optimal incubation time of EZ-LiFT Reagent for this human iPS culture.

Too long of EZ-LiFT incubation time: Cell clumps dissociate into single cell suspension.

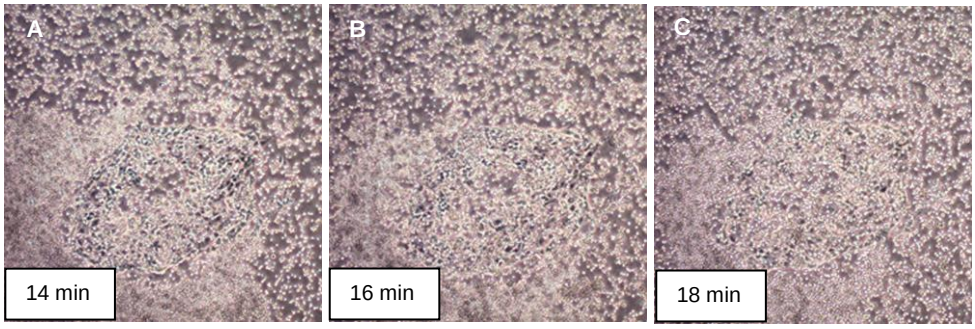

Figure 3. Human iPS colonies dissociate into single cell suspension if left too long in EZ-LiFT Reagent (**A-C**). For this particular human iPS line, 14-18 minutes EZ-LiFT incubation was too long.

EZ-LiFT can rescue highly differentiated hES/iPS cultures.

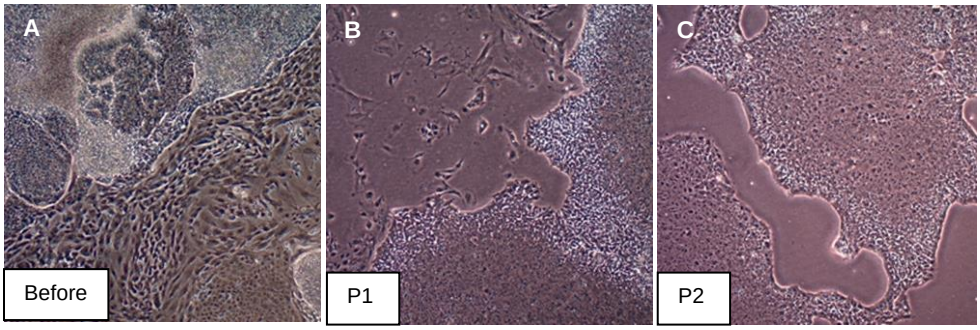

Figure 4. Human iPS cells derived from PBMCs were allowed to spontaneously differentiate (~60% differentiation) before passaging with EZ-LiFT Reagent (**A**). Six to 8 days after the first passage, the culture contains mostly undifferentiated pluripotent colonies with less than 5% differentiated cells observed (**B**). By the 2nd passage with EZ-LiFT, culture is ~100% undifferentiated pluripotent cells (**C**).