hPSC Dissociation Buffer

Product Manual

I. Product Introduction

Shownin hPSC Dissociation Buffer (RP01007) contains 0.5mM EDTA. The dissociation buffer is filtered for sterilization and can be directly used for the dissociation of pluripotent stem cells. hPSC Dissociation Buffer is convenient and efficient. Typically dissociating most pluripotent stem cells in about 8 minutes at 37°C.

II. Product Information

Table 1: hPSC Dissociation Buffer Product Description

Product Information	Cat.No.	Amount	Concentration
hPSC Dissociation Buffer	RP01007	500 mL	0.5 mM EDTA

III. Storage Conditions

1. Storage temperature: 2-8℃.
2. Shelf life: 12 months.

IV. Instructions

1. Aspirate the culture medium from the hPSC wells, add 2 mL/well of DPBS (without calcium and magnesium). Gently shake and aspirate.
2. Add 2 mL/well of hPSC Dissociation Buffer. Ensure the solution completely covers the bottom of the wells.
3. Culture the cells in a 37°C incubator for 7-8 min.

TIPS: (1) After 8 minutes of dissociation, observe the cell changes under the microscope. When most cells become brighter and rounder, and cells have not yet detached from the matrix or floated up, terminate the dissociation (Figure 1C). If most cells have not yet become brighter, extend the dissociation (Figures 1A&B).
(2) Keep the 6-well plate in direct contact with the metal partition of the incubator to ensure uniform heating of the plate. Do not stack the plates.

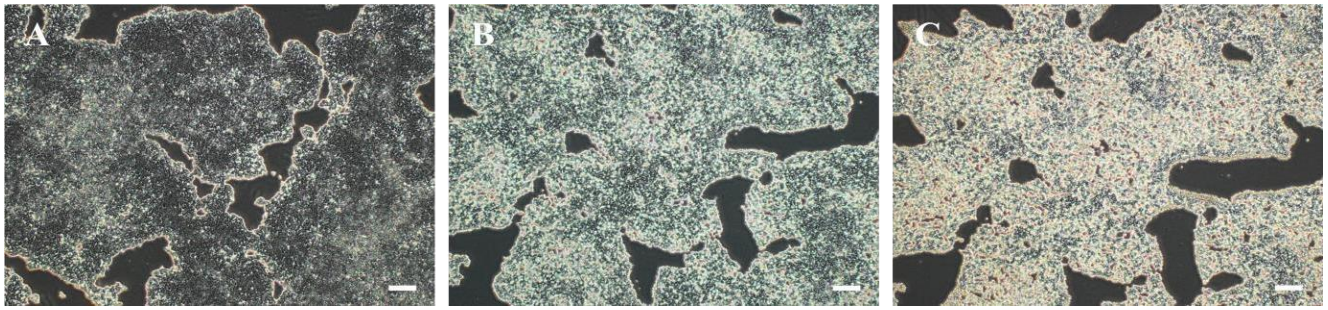

Figure 1: Example images of hPSC dissociation using hPSC Dissociation Buffer.

(A) dissociation for 4 min; (B) dissociation for 6 min; (C)dissociation for 8 min. Scale bar: 200 μm.

4. After dissociation, gently bring the cell culture plate back to the biosafety cabinet. Avoiding shaking the cells by vibration. Tilt the plate to aspirate the hPSC Dissociation Buffer.
5. Promptly add 2 mL/well of pre-warmed hPSC complete medium containing ROCK inhibitors (NcEpic or NcTarget, Blebbistatin 2.5 μM or Y27632-2HCL 10 μM). Shake the 6-well plate horizontally in a cross pattern to dislodge the cells from the substrate.

   TIPS: (1) When adding hPSC complete medium containing ROCK inhibitor (NcEpic or NcTarget), gently pipette the cells 1-2 times. Do not pipette more than 2 times. Avoid dispersing the cells into a single-cell state.
   (2) Avoid scraping the cells, it is normal for some cells (10-15%) to remain attached to the matrix. If a large number of cells are not detached, extend the dissociation time.
   (3) Do not operate more than one 6-well plate at a time. Aspirate quickly after adding hPSC complete medium (NcEpic or NcTarget). The effect of the hPSC Dissociation Buffer is quickly terminated after the addition of hPSC complete medium, while the cells will quickly reattach. Do not treat hPSCs with the hPSC Dissociation buffer for an extended period (<15 min). Therefore, the collection and re-seeding of cells must be done quickly.

6. Seeding:
   6.1 Aspirate the coating solution from Matrigel or Vitronectin-coated 6 well culture plates. Add 2 mL/well of pre-warmed complete medium containing ROCK inhibitors (Blebbistatin 2.5 μM or Y27632-2HCL 10 μM).
   6.2 Gently shake the cell suspension obtained in Step 5 to mix it evenly, and then distribute the cells evenly into the 6-well plate according to the pre-set sub-culturing ratio.

   TIPS: Alternatively, calculate the required number of cells for each passage per plate. Transfer the cells to a 15 mL centrifuge tube. Resuspend them in pre-warmed complete culture medium containing ROCK inhibitor (Blebbistatin 2.5μM or Y27632-2HCL 10μM) to a final volume of 12 mL. Evenly distribute the suspension to 6-well plate from which the coating solution has been aspirated.

7. Gently rock the 6-well plate horizontally in a cross pattern three times. Bring it to the incubator and rock it again in the cross pattern three more times. Culture overnight at 37°C with 5% CO2 concentration and saturated humidity.
8. Replace the medium with fresh hPSC complete medium (NcEpic or NcTarget) after 18-24 hours. Change the medium daily thereafter. Passage or harvest cells after 4-5 days.