hPSC Cryopreservation Medium

Catalog # SN-06-1210 50 mL

Product Introduction

The hPSC Cryopreservation Medium is a human pluripotent stem cell (hPSC) cryoprotective solution with defined chemical composition, serum-free formula, and no animal-derived components. This product is specially formulated for hPSC cryopreservation, which can greatly reduce the damage of cells in the process of cryopreservation, improve the survival rate of cells after recovery, and effectively maintain the multidirectional differentiation potential of hPSC in the long term. Meanwhile, the chemical composition of the cryopreservation solution is clear, without exogenous protein components, and the quality is stable from batch to batch, so it is suitable for scientific research-grade cell preservation.

Product Information

Table 1. hPSC Cryopreservation Medium Description

Product	Amount	Cat.No.	Storage
hPSC Cryopreservation Medium	50 mL	SN-06-1210	2℃～8℃

Storage Conditions

1. Storage temperature: 4°C.

2. Shelf life: 12 months.

Cryopreservation of hPSCs (using a 6-well plate as an example, the procedure also applies to other culture vessels)

1. When the cell confluence reaches approximately 85%, cells can be harvested for cryopreservation. Typically, a 6-well plate can collect 2–4 × 10^6 viable cells per well, sufficient for one cryovial.

2. Prepare the appropriate number of 1.5/2 mL cryovials.

3. Retrieve the hPSC Cryopreservation Medium from the 4°C refrigerator and allow it to equilibrate to room temperature. **Shake gently before use.**

4. Aspirate the spent hPSC culture medium, then add 2 mL/well of DPBS (without calcium or magnesium) and gently shake a few times. Aspirate again.

5. Add 2 mL/well of hPSC Dissociation Buffer, and incubate the cells in a 37°C incubator for 7–8 minutes.

6. After digestion, gently remove the culture plate and aspirate the hPSC Dissociation Buffer.

7. Gently mix the pre-warmed hPSC Cryopreservation Medium . Add 1 mL of hPSC Cryopreservation Medium per well. Gently pipette the solution, shake in a horizontal cross-pattern three times, then transfer the cell suspension into the 1.5/2 mL cryovials.

8. Place the cryovials in a controlled-rate freezing container and store in a -80°C freezer overnight. The following day, transfer the cryovials to a liquid nitrogen storage tank for long-term storage. Alternatively, use a programmable freezing device to cool the cells below -80°C and then transfer directly to liquid nitrogen storage.

Thawing hPSC (using a 6-well plate as an example; the procedure also applies to other culture vessels)

1. Prewarm a water bath to 37°C.

2. Place a Vitronectin-coated 6-well plate in a biosafety cabinet for approximately 1 hour to equilibrate to room temperature (15–30°C).

3. Prepare 4 mL of complete hPSC medium (NcEpic or NcTarget). Add 1 µL of Blebbistatin (10 mM) at a 1:4000 dilution. Allow the medium to equilibrate to room temperature (15–30°C).

   Tips: Do not prewarm the medium in a 37°C water bath.

4. Retrieve one cryovial of frozen cells and gently agitate it in a 37°C water bath. Thaw within 1 minute. Remove the vial when ice crystals are nearly invisible.

5. Wipe the cryovial with 75% ethanol using a lint-free wipe and transfer it to a biosafety cabinet. Transfer the cell suspension to a pre-labeled 15 mL centrifuge tube. Slowly add 10 mL of DMEM/F12 dropwise while gently mixing. Centrifuge at 160 × g for 5 minutes.

6. Discard the supernatant. Resuspend the pellet in 4 mL of pre-warmed complete hPSC medium (NcEpic or NcTarget) containing Blebbistatin. Avoid pipetting up and down to minimize cell stress.

7. Aspirate the Vitronectin coating solution from two wells of the 6-well plate. Seed 2 mL of the cell suspension into each well.

8. Gently shake the plate in a crosswise motion three times, place it in a 37°C, 5% CO_2, humidified incubator, and shake again three times in the same manner.

9. Replace the medium with fresh complete hPSC medium (NcEpic or NcTarget) after 18–24 hours. Subsequently, perform daily medium changes.