Instructions for Using MSC Proliferation Culture Medium KIT

❖ Preparation:

1. Reagent preparation: prepare the reagent according to the proportion of 500ml culture medium special for MSC Proliferation(Xeno-free, no need for coating) added to 25ml MSC growth additiveand mix well for use.

2. **Cells:** Primary mesenchymal stem cells (umbilical cord, placenta, amniotic membrane, fat, dental pulp, etc.) obtained by tissue block adhesion or enzyme digestion, human umbilical cord mesenchymal stem cells (UC MSCs) cultured with serum or serum substitutes, placental MSCs and adipose mesenchymal stem cells (ADSCs), as well as deeply cryopreserved umbilical cord mesenchymal stem cells, placental mesenchymal stem cells, and adipose mesenchymal stemcells, etc.

3. **Adherent Culture** (37°C, 5%CO2)。

❖ Operation Procedure: (Attention: This reagent is for research purposes only.)

A. Primary Cell Culture:

Inoculate the digested primary cells with a live cell count of 2x10^4 cells/cm² into a T75 culture bottle. Add 25ml of serum free medium to T75 culturebottleand place it in a 37 ℃, 5% CO_2 incubator. Change the solution every 3 days, and when the cell fusion degree in the bottle reaches 70-80%, the cells can be digested and collected (400×g Centrifuge for 5 minutes).

B. Passage Cell Culture:

The passage cells are inoculated with (0.8-1.2x10^4 cells/cm2) into a T175 (or T75) culture flask. Add 25ml (T75 with 15ml) of serum free culture medium. Incubate in a 5% CO2 incubator at 37 °Cwithout changing the medium during the process. Once the cell fusion degree reaches 80% -90%, it can be passaged.

❖ Cryopreservation and Recovery:

Frozen cultured cells are needed to be resuspended into MSC cryopreservation solution according to the standard of 1 × 10 ^6-1 × 10 ^7cells/ml. Then cool them to -80°C and store them in -196 °C liquid nitrogen next day (note: some cryopreservation solutionsdo not require programmed cooling).

Cells frozen in liquid nitrogen quickly melt in a 37 °C water bath until they contain a small amount of ice water mixture, and then inhale into a 50ml centrifuge tube. Slowly add 10-20 ml of serum-free culturemedium along the tube wall, and gently mix the liquidinside the tube while adding.

Then at room temperature, operate the 400×g Centrifuge for 5 minutes to discard the supernatant, and add an appropriate amount of serum-free culturemedium to adjust cell density.

Finally, inoculate T175(or T75) culture bottle with a live cell count of 0.8-1.2x10^4 cells/cm²(microcarrier inoculation density of 0.6-0.8x10^4cells/cm²), and add 25ml (T75 to 15ml) of serum-free culture medium.

Incubate in a 5% CO_2 incubator at 37 °C (without changing the medium throughout the process) until the cellfusion degree reaches 80% -90% when the cells can be passaged.

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