Instructions for Using MSC Culture Medium KIT

❖ Preparation:

1. Coating agent preparation: Add the coating reagent into sterileDPBS to make 0.5% (v/v) coating working solution and mix well.
2. Media carrier coating: According to the area of the media carrier, add coating solution at the ratio of 0.1mL/cm2 and shake gently to make the coating solution spread evenly on the bottom of the bottle. Place the bottle horizontally in the refrigerator at 4°C to keep it away from light, and it can be used 12~18 hours after coating (it can be kept in the refrigerator at 4°C for one week after coating). For emergency use, store at 37°C for 2 hours before use.
3. Enhancer Preparation: Add 30 ml of enhancer to 500 ml of MSC serum-free medium for cell culture, store at 2-8°C and use up within 1 month.

❖ 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 20000 cells/cm^2 into a T75 culture bottle coated with this enhancer.Add 25ml of serum free medium to T75 and 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 (8000-12000 cells/cm2) into a T175 (or T75) culture flask coated with this enhancer. Add 25ml (T75 with 15ml) of serum free culture medium. Incubate in a 5% CO_2 incubator at

37 ℃ without 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 × 106-1 × 107cells/ml. Then cool them to -80°C and store them in -196 °C liquid nitrogen next day (note: some cryopreservation solutions do not require programmed cooling).

Cells frozen in liquid nitrogen quickly melt in a 37 ℃ 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 culture medium along the tube wall, and gently mix the liquid inside 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 culture medium to adjust cell density.

Finally, inoculate the pre- coated T175(or T75) culture bottle with a live cell count of 8000-12000 cells/cm^2(microcarrier inoculation density of 6000cells-8000cells/cm^2), and add 25ml (T75 to 15ml) of serum-free culture medium.

Incubate in a 5% CO_2 incubator at 37 ℃ (without changing the medium throughout the process) until

the cellfusion degree reaches 80% -90% when the cells can be passaged.

Add: No.128, Huatuo Road, Hailing District, Jiangsu, China Tel: 0086523-86209201 86209158 86209168