



## Xcell Therapeutics

---

### Head Office (SEOUL)

06188  
Dongwon Bldg. 6F, 333, Yeongdong-daero,  
Gangnam-gu, Seoul, Korea

**T.** +82-2-863-1331  
**F.** +82-2-863-0832  
**E.** [info@xcell.co.kr](mailto:info@xcell.co.kr)  
[ts@xcell.co.kr](mailto:ts@xcell.co.kr)

### GMP Facility (YONGIN)

16954  
#1908, 13, Heungdeok 1-ro, Giheung-gu  
Yongin-si, Gyeonggi-do, Korea

**T.** +82-31-214-1088  
**F.** +82-31-660-7058  
**E.** [gmp@xcell.co.kr](mailto:gmp@xcell.co.kr)



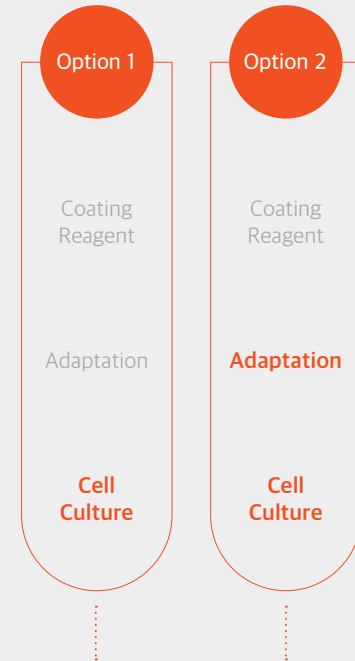
# CONTENTS

- Product Information**
1. Product Description
  2. Intended Use
  3. Safety Information

- Medium Preparation**
1. Preparation
  2. Storage

- Cell Culture**
1. Thawing
  2. Subculture
    - Protocol Options\*
    - Op1. Basic Protocol: Easy to Use
    - Op2. Adaptation: Improves culture stability (when replacing the medium)
  3. Cryopreservation

## \* Protocol Option



Requirements	-	Additional Adaptation Step
Benefits	Simple Culture Method	Stable Cell Culture Environment

## CellCor™ CD MSC

Product	Catalog #	Size	Storage	Shelf Life*
CellCor™ CD MSC	YSP002	500 mL	Under -20°C	Expiry date on label

\* Stable for 12 months from the Date of Manufacture.

### 1. Product Description

CellCor™ CD MSC is a serum-free chemically defined medium for the expansion and growth of human mesenchymal stem cells (hMSCs). CellCor™ CD MSC consists only of synthetics or recombinant proteins and is free from human/animal derived lysates or extracts. Quality control tests such as Bioactivity, Endotoxin, and Mycoplasma are conducted.

#### NOTES

- ※ Does not contain antibiotics.
- ※ Coating agents may be used if needed.

### 2. Intended Use

For Research and Further Manufacturing Use

- ※ Not intended for direct administration into humans or animals.
- ※ Not intended for in vitro diagnostics (IVD).

### 3. Safety Information

Read and follow the instructions on the Material Safety Data Sheet (MSDS).

Be sure to wear appropriate protective clothing, eyewear, and gloves.



### 1. Preparation

- ① Conduct a visual inspection for any damage, change in color, and thawing of the product.
  - Picture of the product.
    - ※ To check if any damage during the shipping process
- ② Thaw CellCor™ CD MSC at room temperature for 1 hour, then completely thaw in a 37°C water bath.
  - Medium has been completely thawed.
    - ※ In case the medium has not been completely thawed, place in a water bath until no residues are left.
  - Before and After picture of the medium.

### 2. Storage

- ① Thawed CellCor™ CD MSC is stable for up to 4 weeks when stored at 4°C.
- ② When using the remaining CellCor™ CD MSC (stored at 4°C), aliquot and warm the medium in a 37°C water bath for 30 minutes.
  - ※ Recommended using immediately after thawing.
  - ※ Avoid repeated freeze-thaw cycles.



# CHECK LIST

Check the results according to the experiment below

## 1. Thawing Process

### Experimental process

1. Disinfect the interior of a BSC(Biosafety Cabinet) with 70% ethanol, and prepare warmed CellCor™ CD MSC. Add 9 mL of pre-warmed CellCor™ CD MSC into a 15 mL tube.
2. Thaw cryovial in a 37°C water bath for 1-2 minutes.
3. Disinfect the surface of the cryovial with 70% ethanol and place it in the BSC.
4. Carefully transfer 1 mL of thawed cells to the 15 mL tube containing 9 mL of CellCor™ CD MSC.
5. Centrifuge the tube at 230 x g, 20°C for 3 minutes.
6. Remove the supernatant, resuspend the cell pellet with CellCor™ CD MSC, and count cells.
7. Seed a T75 Flask at a density of 4,000-5,000 cells/cm<sup>2</sup> with 15 mL of CellCor™ CD MSC.
8. Incubate at 37°C in a humidified atmosphere of 5% CO<sub>2</sub> (3 ~ 4 days on average).
- 9-1. (For 3 ~ 4 days of culture) Subculture the cells when cell confluency reaches 75-85%. (Figure 1)
- 9-2. (For more than 4 days of culture) Change the medium on the 4th day of culture. Subculture the cells when cell confluency reaches 75-85%. (Figure 1)

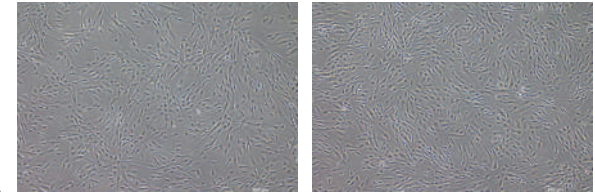


Figure 1.  
Cell Confluency

75%

85%

### Results

Cell Type : \_\_\_\_\_ , Passage : \_\_\_\_\_

Centrifuge Condition : \_\_\_\_\_ g or \_\_\_\_\_ rpm, \_\_\_\_\_ °C, \_\_\_\_\_ min

Seeded cell density : \_\_\_\_\_ cells/cm<sup>2</sup>

※ Compared to a medium containing FBS or hPL, CellCor™ CD MSC should be cultured with an appropriate number of cells (about 30% confluent). The growth of cells may be affected by the number of seeding.

Cell culture period : \_\_\_\_\_ days

※ Do not change the medium until subculture of thawed cells.

Cell morphology before subculture (Confluency 75~85%) (Picture)

Cell morphology after medium change (after 1 day) (Picture)

Cell morphology before subculture (Confluency 75~85%) (Picture)



\*Protocol Option  
Op.1) Basic Protocol

[1] Subculture

Experimental process

1. Remove the cultured medium from the T75 Flask and wash with 7 mL of DPBS.
2. Add 2 mL of TrypLE™ Express (Gibco, Catalog #12604013) into the flask to evenly distribute the reagent. Incubate the cells at 37°C for 3 minutes.
3. Observe under a microscope to check for cell detachment.
4. Collect the cells with 4 mL of CellCor™ CD MSC and transfer to the tube (Repeat once).
5. Centrifuge tube at 230 xg, 20°C for 3 minutes.  
※ Eliminate every possible Detachment Reagent (e.g., TrypLE™ Express etc) from the centrifuged tube.
6. Remove the supernatant, resuspend the cells with CellCor™ CD MSC and determine the viable cell density using a preferred method.
7. Seed a T75 flask with 4,000-5,000 cells/cm<sup>2</sup> with 15 mL of CellCor™ CD MSC.
8. Incubate at 37°C in a humidified atmosphere of 5% CO<sub>2</sub> (3 ~ 4 days on average)
- 9-1. (For 3 ~ 4 days of culture) Subculture the cells when cell confluency reaches 75-85%. (Figure 1)
- 9-2. (For more than 4 days of culture) Change the medium on the 4th day of culture. Subculture the cells when cell confluency reaches 75-85%. (Figure 1)

CHECK LIST

Check the results according to the experiment below

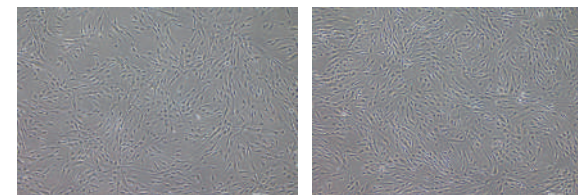


Figure 1.  
Cell Confluency

75%

85%

Results

- Cell Type : \_\_\_\_\_ , Passage : \_\_\_\_\_
- Cell morphology after subculture (after the attachments, approximately 3hrs) (Picture)
- Used detachment Reagent type : \_\_\_\_\_  
※ Using chemically defined reagents compliant with the CD environment is recommended.  
※ Use mild reagents (e.g., TrypLE™ Express etc) is recommended since there are no components in CellCor™ CD MSC that can neutralize Trypsin enzyme.
- Observation under a Microscope (Picture)  
※ Gently tap the flask when cells do not detach.
- Centrifuge Condition : \_\_\_\_\_ g or \_\_\_\_\_ rpm, \_\_\_\_\_ °C, \_\_\_\_\_ min
- Seeded cell density : \_\_\_\_\_ cells/cm<sup>2</sup>  
※ Compared to a medium containing FBS or hPL, CellCor™ CD MSC should be cultured with an appropriate number of cells (about 30% confluent). The growth of cells may be affected by the number of seeding.
- Cell culture period : \_\_\_\_\_ days
- Cell morphology before subculture (Confluency 75~85%) (Picture)
- Cell morphology after medium change (after 1 day) (Picture)
- Cell morphology before subculture (Confluency 75~85%) (Picture)



\*Protocol Option  
**Op.2) Adaptation**

## CHECK LIST

Check the results according to the experiment below

### Option

#### Experimental process

Following the Adaptation Protocol is recommended when using CellCor™ CD MSC for the first time to maintain stabilized cell conditions.

### [1] Adaptation

#### Experimental process

1. After thawing or culturing the cells for 24 hours, discard half of the previous medium and add the same amount of CellCor™ CD MSC.
2. Incubate at 37°C until cell confluency reaches 75~85% (3~4 days).

#### MEMO

#### Results

- Follow Adaptation Protocol: ( Y / N )

#### Results

- Cell morphology in the former medium (Picture)  
※ i.e. In a T-75 flask, Culture with 15 mL of the previous medium for 24 hours. Remove 7.5 mL of the former medium and add 7.5 mL of CellCor™ CD MSC.  
※ The Adaptation Protocol is recommended to stabilize cell culture conditions.
- Cell morphology after cell adaptation  
※ Cell morphology may temporarily change during the adaptation phase.



\*Protocol Option  
Op.2) Adaptation

[2] Subculture

Experimental process

1. Remove the cultured medium from the T75 Flask and wash with 7 mL of DPBS.
2. Add 2 mL of TrypLE™ Express (Gibco, Catalog #12604013) into the flask to evenly distribute the reagent. Incubate the cells at 37°C for 3 minutes.
3. Observe under a microscope to check for cell detachment.
4. Collect the cells with 4 mL of CellCor™ CD MSC and transfer to the tube (Repeat once).
5. Centrifuge tube at 230 xg, 20°C for 3 minutes.  
※ Eliminate every possible Detachment Reagent (e.g., TrypLE™ Express etc) from the centrifuged tube.
6. Remove the supernatant, resuspend the cells with CellCor™ CD MSC and determine the viable cell density using a preferred method
7. Seed a T75 flask with 4,000-5,000 cells/cm<sup>2</sup> with 15 mL of CellCor™ CD MSC.
8. Incubate at 37°C in a humidified atmosphere of 5% CO<sub>2</sub> (3 ~ 4 days on average)
- 9-1. (For 3 ~ 4 days of culture) Subculture the cells when cell confluency reaches 75-85%. (Figure 1)
- 9-2. (For more than 4 days of culture) Change the medium on the 4th day of culture. Subculture the cells when cell confluency reaches 75-85%. (Figure 1)

CHECK LIST

Check the results according to the experiment below

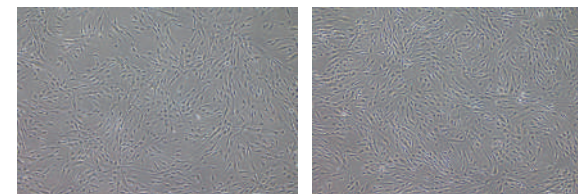


Figure 1.  
Cell Confluency

75%

85%

Results

- Cell Type : \_\_\_\_\_ , Passage : \_\_\_\_\_
- Cell morphology after subculture (after the attachments, approximately 3hrs) (Picture)
- Used detachment Reagent type : \_\_\_\_\_  
※ Using chemically defined reagents compliant with the CD environment is recommended.  
※ Use mild reagents (e.g., TrypLE™ Express etc) is recommended since there are no components in CellCor™ CD MSC that can neutralize Trypsin enzyme.
- Observation under a Microscope (Picture)  
※ Gently tap the flask when cells do not detach.
- Centrifuge Condition : \_\_\_\_\_ g or \_\_\_\_\_ rpm, \_\_\_\_\_ °C, \_\_\_\_\_ min
- Seeded cell density : \_\_\_\_\_ cells/cm<sup>2</sup>  
※ Compared to a medium containing FBS or hPL, CellCor™ CD MSC should be cultured with an appropriate number of cells (about 30% confluent). The growth of cells may be affected by the number of seeding.
- Cell culture period : \_\_\_\_\_ days
- Cell morphology before subculture (Confluency 75~85%) (Picture)
- Cell morphology after medium change (after 1 day) (Picture)
- Cell morphology before subculture (Confluency 75~85%) (Picture)



1. Prepare pellet, resuspend with 1mL of cryopreservation solution (e.g., CELLBANKER 2, amsbio, Catalog #11891), and transfer to a cryovial.
2. Immediately place the cryovials in a freezing container and place overnight at  $-80^{\circ}\text{C}$ .
3. Transfer the cryovials into LN2 tank after 24 hours.



CellCor™ is a chemically defined medium specifically for cell culture.  
With CellCor™ CD MSC as our first step,  
Xcell Therapeutics is constantly developing our product lines and services.