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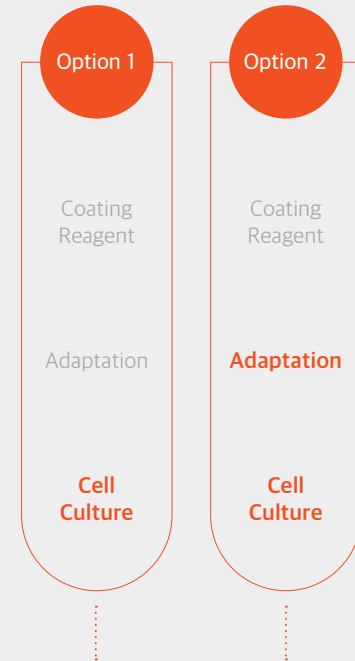
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* Protocol Option



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 synthetic 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/cm² with 15 mL of CellCor™ CD MSC.
8. Incubate at 37°C in a humidified atmosphere of 5% CO₂ (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)

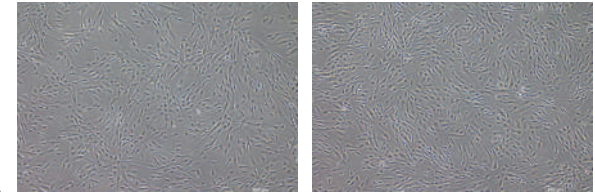


Figure 1.
Cell Confluency

75%

85%

Results

Cell Type : _____ , Passage : _____

Centrifuge Condition : _____ g or _____ rpm, _____ °C, _____ min

Seeded cell density : _____ cells/cm²

※ 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/cm² with 15 mL of CellCor™ CD MSC.
8. Incubate at 37°C in a humidified atmosphere of 5% CO₂ (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)

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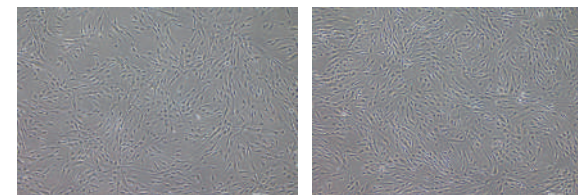


Figure 1.
Cell Confluency

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/cm²
※ 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/cm² with 15 mL of CellCor™ CD MSC.
8. Incubate at 37°C in a humidified atmosphere of 5% CO₂ (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)

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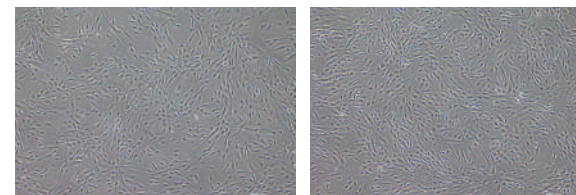


Figure 1.
Cell Confluency

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/cm²
※ 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°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.