

For Further Manufacturing and Laboratory Use

# **SUPERGROW Cell Culture Supplemental Mix**

Cat#: 6122011/6122012

# [Product Name]

SUPERGROW Cell Culture Supplemental Mix

### [Product Description]

SUPERGROW Cell Culture Supplemental Mix (SGR-SM) is a serum substitute suitable for the culture of lymphocytes in vitro and is rich in a large number of growth factors, cytokines and proteins, that provide natural nutrient for cell expansion culture. This product does not contain nor use any primary raw materials that are derived directly from non-human animal during manufacturing process. The product SGR-SM has stable inter-batch quality. It can be used to culture various cells such as DC, CIK, NK and CTL in combination with diverse serum-free media. During use, add to the media according to a certain concentration ratio.

### [Model & Specification]

Product Name	Cat. No.	Size
SUPERGROW Cell Culture Supplemental Mix	6122012	25 mL/bottle
	6122011	250 mL/bottle

#### [Medium Preparation]

1. Prepare 975 mL of serum-free medium and restore it to room temperature.

2. Prepare 25 mL of SGR-SM and thaw in a water bath at 37°C; centrifuge  $3400 \times g$  for 5 minutes and take the supernatant or filter directly with a 40  $\mu$ m cell strainer.

3. Add centrifuged or filtered SGR-SM to the serum-free medium as needed.

4. The prepared complete medium can be stored at 2°C~8°C away from light for 2 weeks.

# [CIK Cell Culture] (in combination with Induced Killer Cells Induction Culture Kit Cat#: 6111511)

1. On Day 0, inoculate the separated human peripheral blood mononuclear cells (PBMCs) into the culture flask (inoculum density recommended as  $2 \times 10^6$  cells/mL) using complete medium containing 2.5% SGR-SM (thawed in water bath at 37°C and filtered with a 40µm cell strainer), add CIK Cytokine I at a ratio of 1%, and culture in a incubator containing at 37°C, 5% CO<sub>2</sub>.

2. On Day 1, add CIK Cytokine II at a ratio of 1%.

3. On Day 3, supplement with an equal volume of fresh medium (containing 2.5% of SGR-SM and 0.05% CIK Cytokine III).

4. From Day 5, take samples for counting every other day, and fill up with fresh cell medium (containing 0.5% of this cell culture supplement and 0.05% CIK Cytokine III) to maintain the cell density at  $1 \times 10^{6}$  cells/mL.

From Day 7 (or when the culture volume is greater than 200 mL), select a larger flask or transfer into the cell culture bag according to the volume of cell culture suspension.

# [NK Cell Culture](in combination with NK Cells Induction Culture Kit 2.0 Cat#: 6813531/6813532/6813533)

1. On Day 0, add a vial of 500µL NK Cell Activator to 1 L of SUPERCULTURE N500 Serum-Free Medium for NK Cells (Cat#: 6113031) to prepare the Amplification Medium for NK cells.

2. Inoculate the separated human PBMCs into the culture flask (density recommended as  $2.5 \times 10^6$  ~ $3.5 \times 10^6$  cells/mL) using the complete medium containing this 10% SGR-SM (thawed in water bath at 37°C and filtered with a 40µm cell strainer), add NK Cell Stimulator (100×), and culture in incubator at 37°C, 5% CO<sub>2</sub>.



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3. On Day 3, supplement with fresh Amplification Medium for NK cells (containing 5% of SGR-SM) at a ratio of 1:1 of the original medium volume to medium volume.

4. From Day 5, take samples every 2 days to count the cell concentration and supplement fresh Amplification Medium for NK cells (containing 2.5% SGR-SM) according to the count results to adjust the cell concentration to  $0.8 \times 10^6 \sim 1 \times 10^6$  cells/mL. (The cell density can be adjusted approximately according to the color change of medium. During cell passage, if the medium is yellowish, the passage density can be adjusted to  $0.8 \times 10^6 \sim 0.9 \times 10^6$  cells/mL; if the medium is reddish, the passage density can be adjusted to  $1 \times 10^6$  cells/mL.)

5. Around Day 7, use a larger flask or transfer into the cell culture bag (recommended use of Cell Culture Bag, Cat#: 6071011/6071012) according to the volume of cell culture suspension. The maximum culture volume of T75 flask is 40 mL and of T175 flask is 200 mL. When the medium volume exceeds 200 mL, transfer into the cell culture bag for culture.

6. The SGR-SM in the fresh Amplification Medium for NK cells supplemented with can be reduced to 1% after Day 7 and 0.5% after Day 11.

# [Storage Conditions and Validity Period]

Keep away from light at-15°C~-25°C, the shelf life is 36 months.

### [Notes]

• Avoid repeated freezing and thawing of this product. Please prepare it into a complete medium as soon as possible after thawing, or freeze at -15°C to -25°C after sub-packaging and be careful to operate aseptically during use.

• Clotting or insoluble particles may form in culture medium during cell culture, which is a reversible clotting phenomenon. It will slowly disappear after cells being put back in the incubator, and has no effect on cell viability, expansion or positive rate.

Product Symbol	Description	Product Symbol	Description
REF	Catalogue number	LOT	Batch code
2	Date of manufacture	Σ	Use-by date
	Manufacturer		Temperature limit
类	Keep away from sunlight		Consult instructions for use

# [Description of Product Symbol]

# [Related Products]

Product Name	Cat No.	Size
L500 Serum-Free Medium for Lymphocyte	6111021	1000 mL/bottle
L500 Serum-Free Medium for Lymphocyte (GMP)	6811021	1000 mL/bottle
CIK Activated Agent Kit (CIK-CYT)	6141011	2×500 μL
L100 Serum-Free Medium for Lymphocyte	6911011	1000 mL/bottle
Recombinant Anti-CD3 Humanized Antibody	6151011	1 mg

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