



## Product Specification

<b>Product name</b>	Lolina® Human BM-MSC Cool-Exo® Enhancer kit 2 (500×), Xeno Free, Exo Plus
<b>Cat.No.</b>	NaC20130302
<b>Storage and shipping</b>	<b>NaC20130302-A:</b> 2-8 °C <b>NaC20130302-B/C/D:</b> Store at -20 °C. Once added to medium, store at 4°C, do not refreeze after thawing. Dry ice transportation. Since the kit contains light-sensitive ingredients, please store it away from light.

## Product Description

Lolina® Human BM-MSC Cool-Exo® Enhancer kit 2 (500×) is a set of sterile powders or concentrated solution which contains growth factors, hormones, or proteins for directed induction of paracrine secretion and exosome protection.

As an treatments additive for BM-MSC in vitro culture, this supplement has been proved has following functions:

1. Strongly active the paracrine secretion of anti-inflammatory exosomes.
2. Effectively avoids cell damage that may occur when anti-inflammatory paracrine secretion is triggered.
3. Enhance the stability of MSCs.
4. The robustness of exosomes can be increased by enhancing the robustness of the exosome membrane.
5. Enhance the stress response pathway within MSCs to improve the robustness of exosomes.

## Components

<b>Compound No.</b>	<b>Compounds</b>	<b>Format</b>	<b>Size</b>
NaC20130302-A	StemExo® Antioxidant reagent	Liquid	2ml
NaC20130302-B	StemExo® AI 1	Lyophilized	500 ×
NaC20130302-C	StemExo® AI 2	Lyophilized	500 ×
NaC20130302-D	r-Human serum albumi (rHSA)	Lyophilized	62.5 mg

## Instructions for Use

## 1. Stock solution Preparation.

One kit is for 50ml cell culture medium.

- a. NaC20130302-A is a ready-to-use reagent, just add it into treatment medium to obtain desired concentration.
- b. NaC20130302-B/C/D are offered as lyophilized powder, their stock solutions are prepared as follows:

The compounds are offered as powder in tubes. Please centrifuge before opening the cap to ensure the accuracy of the dosage.

Please carry out dissolution and packaging operations on a clean bench.

Spray the medium bottle and supplement tube with 70% ethanol and wipe to remove excess liquid. In a sterile field, remove the caps without touching the interior threads with fingers.

Reconstitute **NaC20130302-B/C** in 100  $\mu$ l sterile 1  $\times$  PBS each. Aliquot into appropriate volumes of storage solution. Aliquot into appropriate volumes of storage solution. When stored at -20°C, the stock solution is stable for 6 months. When stored at 4 °C, the stock solution is stable for 1 week.

Reconstitute **NaC20130302-D** in sterile 0.9% NaCl solution. The recommended volume is 5 mL. Aliquot into appropriate volumes of storage solution. When stored at 2-8 °C, -20°C, the stock solution is stable for 24 months.

## 2. Protocol

### Step 1: BM-MSC Culture

**Seeding:** Seed BM-MSC in culture flasks or plates at a density allowing them to reach 70-80% confluence.

**Growth:** Allow BM-MSC to grow until they reach the desired confluence.

### Step 2: Pre-treatment with NaC20130302-A

#### Prepare pre-Treatment Medium:

- a. Add the stock solution of **NaC20130302-A** to the culture medium to a final concentration. The dilution ratio range is from 0.3:50 to 1:13:50.

[Notes]: Please determine the optimal treatment concentration by setting up preliminary experiments to avoid cell damage caused by excessively high concentrations, which may lead to cell detachment and death.

#### Pre-treat BM-MSCs:

- b. Replace the medium by the pre-treatment medium. Treating cells for 2h under standard culture conditions (37 °C, 5% CO<sub>2</sub>).

### Step 3: Treatment BM-MSC with NaC20130302-B/C/D

#### Prepare Treatment Medium:

- c. Add the stock solution of **NaC20130302-B/C/D** to the regular culture medium to obtain a treatment medium. The dilution ratio is: **NaC20130302-B/C** 1:500; **NaC20130302-D** 1:10.

#### Treat BM-MSCs:

- d. Replace the regular culture medium with the treatment medium.

- e. Incubate the BM-MSCs with the treatment medium for 24 hours under standard culture conditions (37 °C, 5% CO<sub>2</sub>).

### **Step 3: Post-Treatment Handling**

- f. **Remove Treatment Medium:** After 24 hours of treatment, remove the treatment medium.
- g. **Wash MSCs:** Wash the cells gently with PBS to remove any residual the treatment medium.

### **Conditioning Phase:**

- h. Replace with fresh, serum-free, or exosome-depleted medium.
- i. Incubate the BM-MSCs for an additional 24-48 hours to collect the conditioned medium containing exosomes.

### **Step 4: Exosome Isolation and Purification**

- j. Collect the conditioned medium after the post-treatment incubation period.

## **Note**

If handled improperly, some components of the medium may present a health hazard. Take appropriate precautions when handling it, including the wearing of protective clothing and eyewear. Dispose of properly.