



## Product Specification

<b>Product name</b>	Lolina® Human AD-MSC Cool-Exo® Enhancer kit 3, Xeno Free, Exo Plus
<b>Cat.No.</b>	NaC20140303
<b>Storage and shipping</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 AD-MSC Cool-Exo® Enhancer kit 3 is a set of sterile powders or concentrated solution which contains growth factors, hormones, or proteins for significant induction of anti-inflammatory paracrine secretion.

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

1. Strongly active the paracrine secretion of anti-inflammatory exosomes. Significantly inhibited the pro-inflammatory factors IL-1 $\beta$  and TNF- $\alpha$  and decreased the relative gene expression of IL-1 $\beta$ , TNF- $\alpha$  and iNOS, while promoting the anti-inflammatory factor IL-10 and increasing the relative expression of IL-10 and Arg-1 in vitro compared with PBS, LPS and Exo groups.
2. By regulating the expression of apoptosis-related genes (such as Bcl-2 and Bax), it inhibits cell apoptosis and promotes cell survival.
3. The robustness of exosomes can be increased by enhancing the robustness of the exosome membrane.
4. Enhance the stress response pathway within MSCs to improve the robustness of exosomes.
5. The generated Exosomes have a significant pro-healing effect on diabetic wounds.

## Components

Compound No.	Compounds	Format	Size
NaC20140303-A	Lipopolysaccharide (LPS)	liquid	100 $\mu$ L
NaC20140303-B	Melatonin	Lyophilized	1.1619 $\mu$ g
NaC20140303-C	r-Human serum albumi (rHSA)	Lyophilized	62.5 mg

# Instructions for Use

## 1. Stock solution Preparation.

One kit is for 50ml cell culture medium.

The compounds are offered as lyophilized powder, their stock solutions are prepared as follows:

- a. The compounds are offered as powder/liquid in tubes. Please centrifuge before opening the cap to ensure the accuracy of the dosage.
- b. Please carry out dissolution and packaging operations on a clean bench.
- c. 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.
- d. **NaC20140303-A** is ready for use, 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 month. When stored at 4 °C, the stock solution is stable for 1 week.
- e. Reconstitute **NaC20140303-B** in 100 µl sterile 1 ×PBS. 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 month. When stored at 4 °C, the stock solution is stable for 1 week.
- f. Reconstitute **NaC20140303-C** in sterile 0.9% NaCl solution. The recommend 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 month.

## 2. Protocol

### Step 1: AD-MSK Culture

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

**Growth:** Allow AD-MSK to grow until they reach the desired confluence.

### Step 2: Treatment AD-MSK with NaC20140303

#### Prepare Treatment Medium:

- a. Add the stock solution of **NaC20140303** to the regular culture medium to obtain a treatment medium. The dilution ratio is: **NaC20140303-A/B** 1:500; **NaC20140303-C** 1:10.

#### Treat AD-MSKs:

- b. Replace the regular culture medium with the treatment medium.
- c. Incubate the AD-MSKs with the treatment medium for 24 hours under standard culture conditions (37 °C, 5% CO<sub>2</sub>).

### Step 3: Post-Treatment Handling

**Remove Treatment Medium:** After 24 hours of treatment, remove the treatment medium.

**Wash MSKs:** Wash the cells gently with PBS to remove any residual the treatment medium.

#### Conditioning Phase:

- a. Replace with fresh, serum-free, or exosome-depleted medium.

- b. Incubate the AD-MSCs for an additional 24-48 hours to collect the conditioned medium containing exosomes.

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

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.