CORE BIOGENESIS

How to work with Core Factors®

1

When You Receive the Products

Ensure the product-containing vials are properly placed in the box -facing upwards-. Otherwise, please contact customer service -information available at product's sheet-.

Take the vials out of the box gently.

Proceed to either storage of the product **or directly to** the next steps in the **reconstitution** and usage protocol.

Recombinant protein as delivered is stable at room temperature for up to 10 days, or frozen at -20 to -80 °C for 6 months.

You can store our products and other valued samples in the provided box. It is Cryo Resistant* ! *up to -80°C.

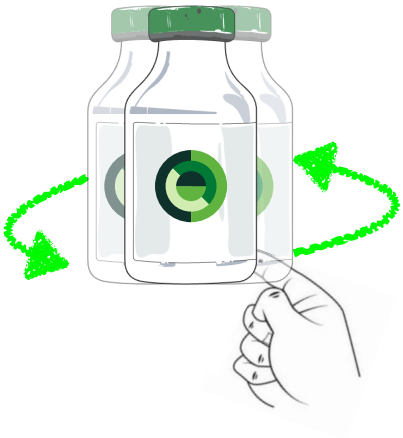
2

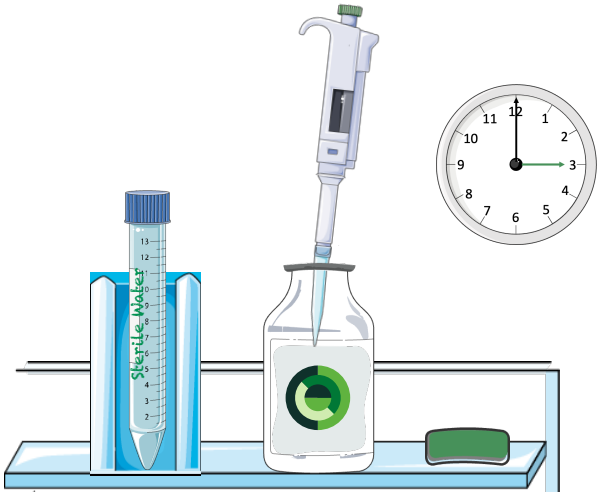
Before Starting Product Reconstitution

Core Factors are supplied in 0.1M Na_2CO_3solution containing the lipid-protein complex.

Proceed to mix the volume content.

Make sure the cap is firmly closed. Take the vials **at RT (15 to 25 °C). Use your fingers to mix throughly the contents by flicking the bottom of the vial.** You can repeat this movement 5-10 times. This will ensure that the lipid-protein complex is properly distributed within the solution, and will prevent that the contents are concentrated at the bottom of the vial, before product reconstitution.





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Reconstitute Protein

Place the vial/s in the class II biological safety cabinet. You can wipe off vials before entering them into the cabinet by using 70% EtOh if vials are appropriately closed.

Remove the cap of the vial by 1st taking out the plastic cover, 2nd peeling off the metallic cap, and 3rd pulling out the rubber stopper -you can also leave the metallic cap and the rubber stopper if you prefer to use a syringe for reconstitution.

*Alternatively, product might be shipped in plastic vials. In this case, you can open the vial by simply removing the cap.

Reconstitute the lipid-protein complex in sterile water or desired buffer.

It is recommended to make at least 0.1mg/mL of stock solution. *Use the growth factor quantity and the indicated volume provided in the product to obtain your desired concentration.

Wait for 5 minutes to allow for optimal reconstitution.

4

Homogenise the Stock Solution

Mix properly the content with the reconstituted protein-lipid complex.

It is recommended to use micropipettes for this process. In addition, you can use low retention pipette tips. This prevents the lipid content from sticking to the wall of the pipette tip.

Throughly mix up and down 10 times with the pipette the whole volume content **until the solution looks homogenous.**

5

Short Term Storage
2 to 8°C for up to 1 week

Long Term Storage
-20 to -80°C for up to 6 months

Aliquot

Prepare single use aliquots to avoid repeated freeze/thaw cycles.

While making aliquots, ensure that the stock solution is still homogeneous. If needed, proceed to additional mixing as indicated in step 4. This will allow preparing aliquots with the same content quantities.

It is recommended to use microtubes with low retention plastic material.

Proceed to store the aliquots following recommended temperatures for short and long term storage. For additional storage information, please refer to the CoA.

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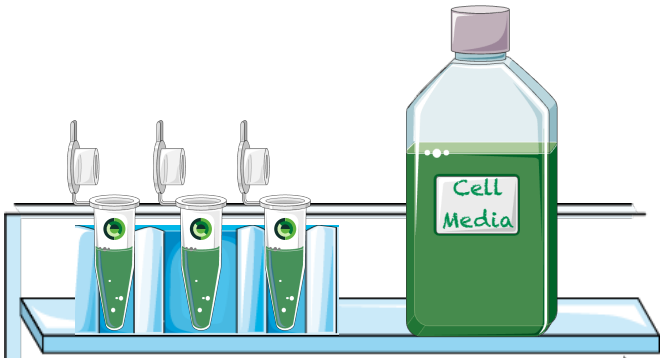
Before Using Core Factors in Cell Culture

Thaw the necessary Core Factors aliquots before making the media preparation with the desired protein concentration.

Preferably, **it is recommended to thaw aliquots in ice** until you observe most of the tube content is in liquid state.

Proceed to mix the aliquot volume up and down 10 times with a micropipette. You can also use low retention pipette tips for this step.



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Cell Media Preparation

Calculate the volume from stock solution aliquots to add into cell culture media, **for obtaining the final desired working concentration** (E.g: for a final 10ng/mL concentration of recombinant protein, you can prepare 50mL media aliquots, by adding 5µl of the stock at 0.1mg/mL)

Work in a class II biological safety cabinet. Once you observe the aliquots have thawed, mix its content up and down gently 5 to 10 times using low retention pipette tips. It is important to avoid bubble formation at this step.

Transfer the corresponding volume into the culture media bottle/tube.

Mix the content throughly to ensure appropriate homogenisation. It is recommended to perform a manual mixing with the bottle/tube closed at this step. Store the Core Factors containing media at 4°C until immediate use.

8

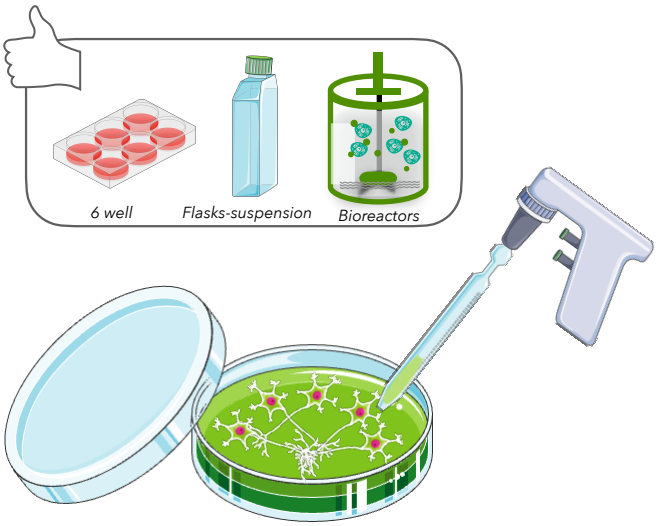
Cell Feeding

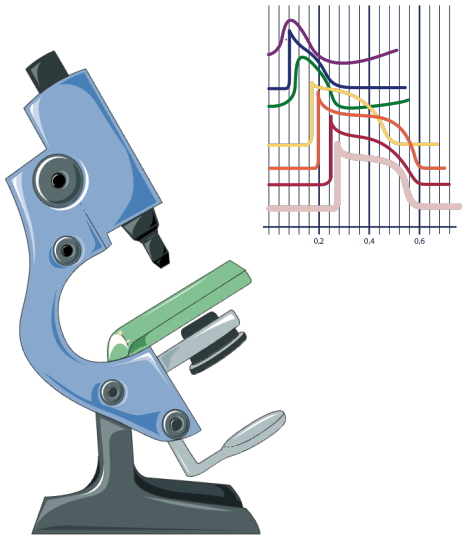
Ensure the Core Factors are evenly distributed within the volume of cell media. Depending on the concentration, Core Factors could be observed by eye with a white-ish colour from its lipid content. If necessary, manually mix 2-4 times the volume of cell media and transfer the bottle/tube into the class II biological safety cabinet.

Re suspend the cell media containing Core Factors 5-10 times with a serological pipette or p1000. Add the appropriate volume into each well/ dish/flask or bioreactor*. Incubate cells at 37°C and 5% of Co2.

We recommend going from 6 well plates, to then scale up your experiments into bigger vessels.

(*) The **bioactivity and performance of Core Factors is optimised for working in 3D and suspension** cell culture conditions.





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Cell Culture Monitoring

Regularly **check the growth and proliferation of your cells under the microscope.** If cells are growing at high densities or occupying a large surface ratio in the culture plate/vessel, it is recommended to make a passage into a bigger culture format. This ensures an appropriate Cell-Core Factor interaction.

Due to the lipid-protein immobilisation complex, the growth factor component of Core Factors remains more stable in culture than soluble proteins. As a result, less media exchange and cell feeding is required.

To determine the optimised replenishment rate of Core Factors, please refer to the stability data available for each protein, define conditions for your cell type, or get in touch with customer service: customers@corebiogenesis.com

Core Factors®

Protocols & Resources