BioExpress® 1000 Preparation for Use

Prior to using the BioExpress® 10x concentrate, it is necessary to dilute the product (1:10 ratio) into a sterile container.

The BioExpress® 10x concentrate is sterile filtered and packaged into bottles that have been autoclaved. However, some customers may wish to sterile filter the media prior to use. CIL provides sterile filtration units with each shipment of 100 mL BioExpress® for this purpose.

The BioExpress® 10x concentrate is shipped to the customer in a box containing two filtration devices. One device is a 500 mL filter unit with an integrated 0.2 micron filter and is meant to be used for preparing larger quantities of media (> 100 - 1000 mL) from the concentrate. The other device is shipped in two parts – a 30 mL syringe and a 0.22 micron filter - and is meant to be used to prepare smaller quantities of media (~ 100 mL) from the concentrate.

All preparation should be performed in a clean environment, preferably in a laminar flow hood to assist in maintaining sterility.

Instructions for Preparing up to 1000 mL Media

1. Use aseptic technique at all times.
2. Dilute the BioExpress® 10x concentrate (1:10) into a suitable container prior to filtration.
3. Use a suitable sterile container to collect the filtered media.
4. Attach the 500 mL filter unit to the sterile container. Wrap with tape to maintain vacuum.
5. Remove the lid from the 500 mL unit.
6. Attach a vacuum line to the hose barb.
7. Pour the diluted BioExpress® into the filter unit and apply the vacuum.
8. Replace the lid to the filter unit.
9. When all the liquid has passed through the filter, remove the vacuum.
10. Remove the filter unit from the container holding the media and place a closure on the container.

Instructions for Preparing 100 mL Media

1. Use aseptic technique at all times.
2. Dilute the BioExpress® 10x concentrate (1:10) into a suitable container prior to filtration.
3. Use a suitable sterile container to collect the filtered media.
4. Filter the diluted media through a 0.22 µm sterile filter unit.
5. Following sterile filtration the media is ready to use.

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BioExpress® 1000 Recommended Testing Protocols

It is not necessary to add glucose or other nutrients to achieve maximal growth. All necessary substrates for optimal growth and protein expression are provided in the media. In order to ensure optimal performance from CIL BioExpress® 1000 the following testing protocol has been developed.

Experiment 1: Growth Curve

Day 1

1. Beginning with a freshly grown plate, choose a single colony of bacteria and inoculate into 2 mL of LB (or other standard media) containing an appropriate antibiotic.
2. Shake tube overnight at the appropriate temperature.

Day 2

1. In the morning, the 2 mL culture should be dense (OD_{600} > 4.0). Inoculate 100 L of this culture into 6 mL each of: A) BioExpress® 1000 and B) Standard Media. (Note: Both media should contain the appropriate antibiotic.)
2. Shake at the appropriate temperature and collect OD_{600} data at the following time points (T_{hours}): T_0, T_2, T_4, T_6, T_8, T_{10}, T_{24} on both cultures for the purpose of comparison.

Experiment 2: Expression Tests

The optimal induction point should now be determined and protein expression can be tested.

1. Repeat experiment 1 exactly and induce (with IPTG, by temperature, with tryptophan, etc.) when the cells reach ca. 50% of the exponential growth phase according to the time established in Experiment 1.
2. At this point (I_0), remove 1 mL of the culture, take an OD_{600} reading, spin and freeze the pellet.
3. Repeat Step 2 at the following time points (I_{hours}): I_2, I_4, I_6, I_{24}.

These pellets should be run on an SDS PAGE gel or have their enzyme activities assayed to compare protein production. If necessary, repeat with larger volumes.

Helpful Hints

1. In general, good aeration is vital to successful growth and expression. Specifically, utilize large (18-25 mm diameter) culture tubes to allow for aeration.
2. When performing Experiment 2, it is critical to repeat identically the details of Experiment 1. For example, the inoculum must come from freshly streaked plates and the same temperature and shaking parameters must be employed.

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