Cell-Free Reaction Reagents – Technical Notes

Reaction Components:

Cap Colour	Minimum volume per tube	Content Description
	112µL or 334µL	**Cell Lysate:** contains enzymes of transcription and translation and metabolic enzymes for energy regeneration. These enzymes are directly collected from a crude *E. coli* lysate.
	112µL	**Solution A:** contains co-factors, NTPs and crowding agents.
	160µL	**Solution B**: contains salts, the 20 canonical amino acids and an energy source.
	50µL	**10mM IPTG:** required when using IPTG-inducible plasmids as template.
	400µL	**Top-up Solution:** optimized solution for topping up the volume of cell free reactions.
	2 x 8µL or 4 x 8µL	**Control DNA:** codes for deGFP with an N-terminal Histidine tag (244 total residues; 27.6kDa) in a cell-free optimized backbone.
	10µL, 20µL, 30µL, 40µL, 50µL, 100µL, 150µL, 200µL, 250µL or 300µL	**100X Linear Reagent:** Enables use of linear DNA as template.

Storage and Handling Instructions:

- Please store the reagents at -80°C as soon as you receive them (positive control DNA and IPTG solution may be kept at -20°C).
- Freeze-thaw cycles will significantly affect the quality of the cell extract. If you are planning on using the kit at multiple time points, we recommend aliquoting and flash freezing the cell extract to minimize activity loss.
- Solutions A and B can be simply be placed back in a -80°C freezer after use, but we strongly recommend flash freezing prior to storage.
- Please thaw all items on ice before use. Vortex solutions A and B once they have thawed. **Do not vortex the cell extract or the DNA**. You can mix the cell extract and DNA by pipetting up and down. Briefly spin down the contents of each tube to collect them at the bottom prior to use. Solution B and Top-up Solution are technically suspensions, so keep spinning them to a minimum.

Application notes:

- Specific yield for cell-free reactions under standard test tube conditions drop as reaction sizes increase. For optimal results, keep reaction sizes below 15 µL in 1.5mL Eppendorf tubes. We provide scale-up solutions as a service.
- Traditional T7-based protein expression plasmids such as pET15 can be used with the cell-free mix. However, the yields are often half of what is possible with an optimized cell-free backbone. If you decide to use a T7-based protein expression plasmids we recommend including 0.5 mM final concentration of IPTG to the cell-free mix.

Protocol:

Here are example reaction setups for typical reaction volumes:

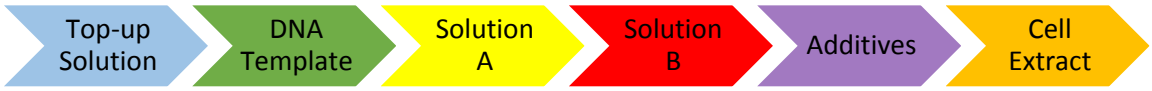
Component	33 µL Reaction	90 µL Reaction	1mL Reaction	Final Concentration
Solution A	3.7 µL	10 µL	111.3 µL	11.1%
Solution B	5.3 µL	14.4 µL	159.7 µL	16.0%
Cell Lysate	11 µL	30 µL	333.3 µL	33.3%
DNA Template	1 µL	2.8 µL	30.3 µL	15nM
Top-up Solution	12 µL	32.8 µL	365.4 µL	Top up to final volume

Incubate the reaction for 16 hours at 26 °C. You can replace the Top-up Solution with nuclease free water or any desired additives. Please make sure to use a high quality DNA prep for your reaction template and elute your DNA in nuclease-free water. Contaminants from DNA purification can highly affect yield (when in doubt, do a PCR clean-up on your template).

If you are using a T7-based protein expression plasmid, the addition of IPTG is necessary. The following is a 1mL reaction set-up for adding 0.5mM final concentration of IPTG from the 10mM stock included in the kit.

Solution	Volume (µL)	Final Concentration
Solution A	111.3	11.1%
Solution B	159.7	16.0%
Cell Extract	333.3	33.3%
DNA Template	30.3	15nM
10mM IPTG	50	0.5mM
Top-up Solution	315.4	Top up to 1mL

For best results, we highly recommend adding the reagents in the following order:



synbio studio

DNA Templates:

This kit is optimized for DNA templates containing a T7 promoter. The architecture and quality of the DNA you use is paramount. We strongly recommend that you use a vector optimized for cell-free expression. An optimized backbone will be provided to you in the kit with the deGFP positive control as insert. The sequence for this template can be made available to you once you purchase one of our kits.

Linear DNA kits are supplied with a 100X Linear Reagent solution. For best results, please include 1µL of this reagent in 100µL of reaction. We recommend using the same design architecture as the optimized control plasmid provided (e.g. promoter, terminator, etc.) for your linear templates.

Performance:

The standard reaction mix can produce over 1.4mg/mL of deGFP from our optimized cell-free expression plasmid. The linear kit enables production of over 1mg/mL of deGFP from optimized linear templates. A pET-15 based backbone can yield 0.8mg/mL of deGFP when induced with 0.5mM IPTG. Approximately 80% of the yield can be achieved in about 6 hours; for higher yields run the reaction for 16 hours.