Cell-Free Reaction Kits – Technical Notes

Kit Contents:

Cap Colour	Minimum Volume (µL)			Content Description
	0.5mL Kit	1mL Kit	5mL Kit	
	1x166.67 or 2x83.33	1x333.34 or 2x166.67 or 4x83.33	5x333.34 or 10x166.67	**Cell Extract:** contains enzymes of transcription and translation and metabolic enzymes for energy regeneration. These enzymes are directly collected from a crude *E. coli* lysate.
	56	112	560	**Solution A:** contains co-factors, NTPs and crowding agents.
	80	160	800	**Solution B**: contains salts, the 20 canonical amino acids and an energy source.
	25	50	250	**10mM IPTG:** necessary for using T7-based protein expression plasmids in cell-free reactions.
	200	400	2000	**Top-up Solution:** optimized solution for topping up the volume of cell free reactions.
	8	16	32	**Control DNA:** codes for deGFP with an N-terminal Histidine tag (244 total residues; 27.6kDa) in a cell-free optimized backbone.

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 gently 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 (Fig. 1). This problem can be solved by using one of our cell-free protein expression cartridges (Fig. 2), which are included in the kit. For small reactions, select a reaction vessel 100X the size of your reaction. For example, a 15µL reaction yields the best results in a 1.5mL tube as opposed to a PCR tube.

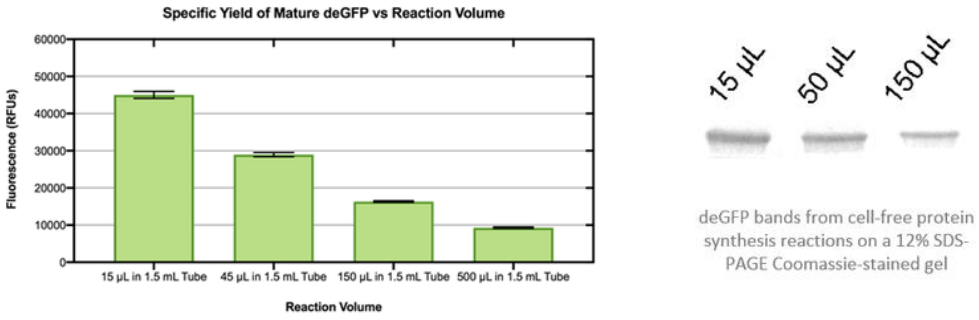


Fig 1. Effect of reaction volume on specific yield under standard test-tube conditions

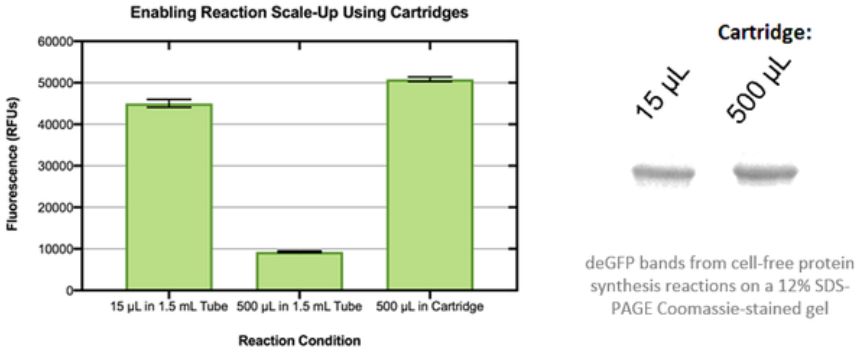


Fig 2. Comparing specific yields with and without cell-free expression cartridges. Here, specific yield for deGFP synthesis in cell-free is demonstrated as an example.

- Traditional T7-based protein expression plasmids such as pET15 can be used with the cell-free mix. However, the yields would be suboptimal (i.e. less than half of what would be possible with an optimized cell-free backbone). In any event, if you decide to use a T7-based protein expression plasmids such as pET15, we recommend including 0.5-1mM final concentration of IPTG to the cell-free mix.

Protocol:

Here is a standard reaction set-up for a 1mL cell-free reaction:

Component	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
Top-up Solution	365.4	Top up to 1mL

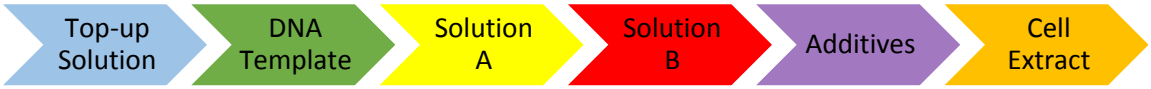
Incubate the reaction for 16 hours at 26.5°C. You can replace the Top-up Solution with nuclease free water or any desired additives. Please make sure to use a high quality DNA prep for your reaction template and elute your DNA in

nuclease-free water. Contaminants from DNA purification can highly affect yield (if 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:



Performance:

The standard reaction mix can produce over 1.4mg/mL of deGFP from our optimized cell-free expression plasmid. 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.