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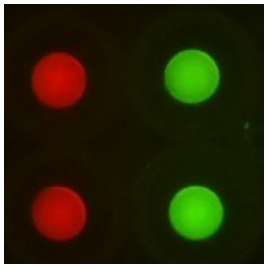
Preparation of cell-free RNAPT7 reactions V.1



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Abstract

This collection contains the protocols and recipes for the preparation of cell-free RNAPT7 transcription/translation coupled reactions.
The crude extracts are obtained from IPTG-treated E.coli BL21 DE3 STAR cells. IPTG triggers RNAPT7 expression and accumulation in cells before collection. Reactions take place when the cell extract is mixed with a maltodextrin-based energy solution along with amino acids solution and other salts (Magnesium and Potassium). This system allows transcription/translation coupled reactions from plasmid DNA that contains T7 promoter transcriptional units. In our hands, it has been possible to express different fluorescent reporters, including deGFP which is highly efficient. Products of the reaction can be measured during the whole reaction time in a plate reader with appropriate optic filters, open hardware cameras, and microscopes. When set up correctlly, deGFP fluorescence intensity is detected as fast as ~30 minutes after the reaction starts and it shows a rapid increase during the first ~2.5-3.0 hours.

The system presented here contains the features highlighted bellow, and is an adaptation of protocols and findings from the following listed papers:

1.- This system uses maltodextrin and Polyphosphate-based energy solution:

Kim, H.-C., Kim, T.-W., Kim, D.-M., . Prolonged production of proteins in a cellfree protein synthesis system using polymeric carbohydrates (2011)

Caschera, F.; Noireaux, V., A cost-effective polyphosphate-based metabolism fuels an all E. coli cell-free expression system. Metabolic engineering (2015)

2.- The S12 crude extract is prepared from E.coli cells (expressing Pol T7) using bead beater.

Sun, Z. Z.; Hayes, C. A.; Shin, J.; Caschera, F.; Murray, R. M.; Noireaux, V., Protocols for implementing an Escherichia coli based TX-TL cell-free expression system for synthetic biology. Journal of visualized experiments (2013)

Kim, TW., Kim, HC., Oh, IS. et al. A highly efficient and economical cell-free protein synthesis system using the S12 extract of Escherichia coli. Biotechnol Bioproc E (2008)

3.- The amino acid stock solution contains each amino acid at ~12 nM in a stable solution:

Caschera, V. Noireaux Preparation of amino acid mixtures for cell-free expression systems. Benchmarks (2015)

Troubleshooting

Files

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Protocol

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Step 3: Performing cell-free RNAPT7 reactions

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Step 1: Preparing S12 cell extracts using bead-beater

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Step 2: Preparing amino acid, polyphosphates, and maltodextrin-based energy solutions for cell-free reactions

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