Jul 01, 2020

Typical Protocol for NEBExpress GamS Nuclease Inhibitor when used with the NEBExpress Cell-free E. coli Protein Synthesis System (NEB #E5360)

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1 *Works for me* This protocol is published without a DOI.

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ABSTRACT

NEBExpress GamS Nuclease Inhibitor is a recombinant protein that inhibits Exonuclease V (RecBCD) activity and stabilizes linear DNA templates in *E. coli* based *in vitro* protein synthesis reactions.

- Enhances synthesis yield of linear DNA using the NEBExpress Cell-free*E. coli*Protein Synthesis System
- Recombinant enzyme; ≥ 95% purity, as determined by SDS-PAGE
- No detectable protease activity, ensuring stability of desired target protein
- Compatible with various target proteins, ranging from 17 to 230 kDa
- Optimal storage buffer for performance in protein synthesis reactions
- Flexible reaction conditions achieve maximum yield; activity can be sustained for 10 hours at 37°C or up to 24 hours at lower temperatures

EXTERNAL LINK

https://www.neb.com/protocols/2019/12/03/typical-protocol--or-nebexpresstm-gams-nuclease-inhibitor-when-used-with-the-nebexpresstm-cell-free-ecoli-protein-synthesis--system-neb-e5360

ATTACHMENTS

E5360 manual.pdf

PROTOCOL CITATION

New England Biolabs 2020. Typical Protocol for NEBExpress GamS Nuclease Inhibitor when used with the NEBExpress Cell-free E. coli Protein Synthesis System (NEB #E5360). **protocols.io** https://protocols.io/view/typical-protocol-for-nebexpress-gams-nuclease-inhi-bfaxjifn

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KEYWORDS

S30 Synthesis Extract , T7 RNA Polymerase , T7 RNA pol, RNase inhibitor , DHFR-His , GamS

CREATED

Apr 19, 2020

LAST MODIFIED

Jul 01, 2020

OWNERSHIP HISTORY

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Jul 01, 2020 New England Biolabs Tech Support New England Biolabs

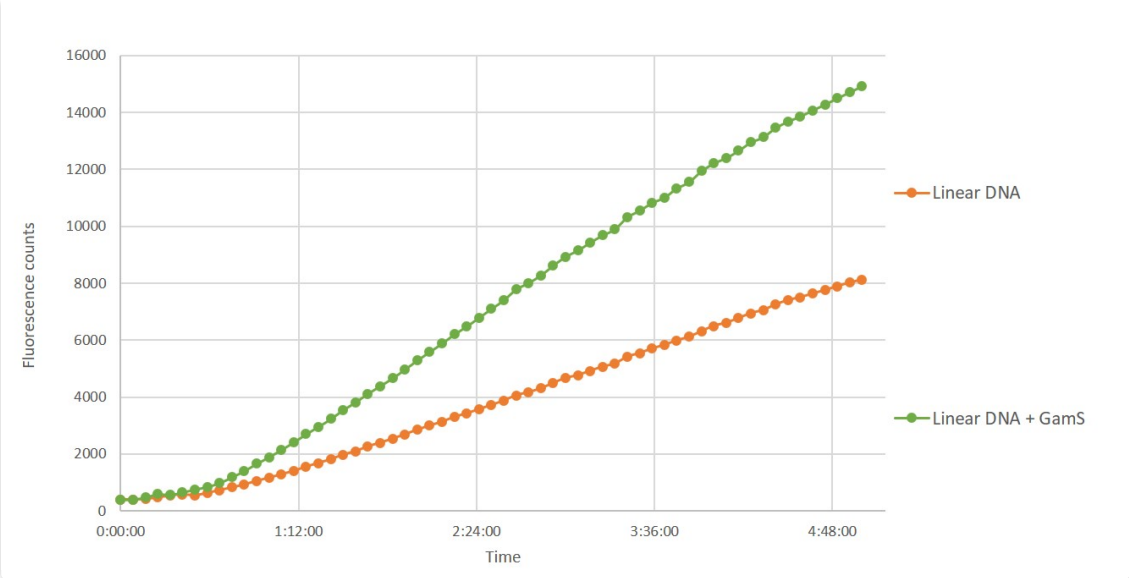
PROTOCOL INTEGER ID
35895

GUIDELINES

NEBExpress GamS Nuclease Inhibitor

NEBExpress GamS Nuclease Inhibitor is a recombinant protein that inhibits Exonuclease V (RecBCD) activity and stabilizes linear DNA templates in *E. coli* based *in vitro* protein synthesis reactions.

Figure 1: Protein synthesis of a linear DNA template using the NEBExpress® Cell-free *E. coli* Protein Synthesis System supplemented with NEBExpress GamS Nuclease Inhibitor



50 µl reactions containing 100 ng linear template DNA, the components of the NEBExpress® Cell-free E. coli Protein Synthesis System and 1.5 µg NEBExpress GamS Nuclease Inhibitor incubated for 5 hours at 37°C were monitored for activity as determined by fluorescence signal.

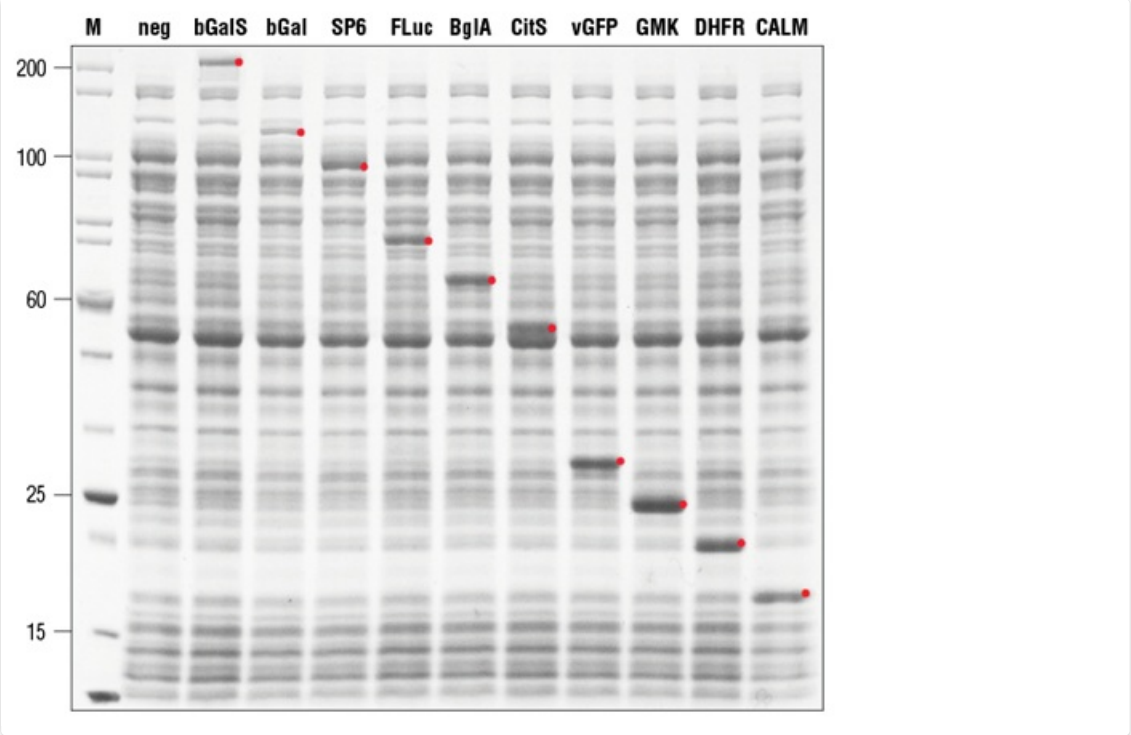
NEBExpress® Cell-free E. coli Protein Synthesis System

The NEBExpress® Cell-free *E. coli* Protein Synthesis System is a coupled transcription/translation system designed to synthesize proteins encoded by a DNA template under the control of a T7 RNA Polymerase promoter. The system offers high expression levels, the ability to produce high molecular weight proteins, scalability, and is cost-effective for high throughput expression applications. The speed and robustness of the system facilitates protein synthesis in applications such as protein engineering, mutagenesis studies and enzyme screening. In addition, it can be used to generate proteins for biophysical and structure-function analyses.

The NEBExpress® Cell-free *E. coli* Protein Synthesis System contains all the components required for protein synthesis, except for the target template DNA. It is a combination of a highly active cell extract from a genetically engineered strain, a reaction buffer, and an optimized T7 RNA Polymerase, which together yield robust expression of a wide variety of protein targets ranging from 17 to 230 KDa.

Protein synthesis is achieved with a short incubation and synthesized protein is compatible with downstream purification or analysis by SDS-PAGE, Western Blot or direct functional assay. The novel formulation of this system allows samples to be loaded directly onto SDS-PAGE, without the need for acetone or TCA precipitation. Additionally, synthesized protein can be isolated from the reaction mixture using affinity purification techniques, such as immobilized metal affinity chromatography (IMAC), for further structural and/or functional characterization.

Figure 1: Protein synthesis using the NEBExpress®Cell-free *E. coli* Protein Synthesis System



50 µl reactions containing 250 ng template DNA were incubated at 37°C for 3 hours. 2 µl of each reaction were analyzed by SDS-PAGE using a 10–20% Tris-glycine gel. The red dot indicates the protein of interest. M = Unstained Protein Standard, Broad range (NEB#P7717), "Neg" = negative control, no DNA.

MATERIALS

NAME	CATALOG #	VENDOR
NEBExpress Cell-free E. coli Protein Synthesis System	E5360L	New England Biolabs
NEBExpress Cell-free E. coli Protein Synthesis System	E5360S	New England Biolabs
NEBExpress GamS Nuclease Inhibitor	P0774S	New England Biolabs

SAFETY WARNINGS

Please refer to the Safety Data Sheets (SDS) for health and environmental hazards.

BEFORE STARTING

Using a positive control template to verify protein synthesis can be useful when unfamiliar with in vitro transcription-translation protocols.

To prevent nuclease contamination, wear gloves and use nuclease-free tubes and tips.

Keep all reagents on ice before and during the assembly of reactions and avoid repeated freeze-thaw cycles of the tubes.

Reactions are typically 50 µl but can be scaled down or up, as needed.

Reactions are typically assembled in nuclease-free 1.5 ml microcentrifuge tubes. Components can be pre-assembled to create a master mix for a desired number of reactions. Use the master mix immediately, discard any unused master mix.

1 Thaw all components **On ice** .

2

Gently vortex the NEBExpress S30 Synthesis Extract and Protein Synthesis Buffer to mix.

3

Combine reagents in a 1.5 ml microcentrifuge tube **On ice** as follows:

COMPONENTS	NEGATIVE CONTROL (no DNA)	POSITIVE CONTROL	SAMPLE
NEBExpress S30 Synthesis Extract	12 µl	12 µl	12 µl
Protein Synthesis Buffer (2X)	25 µl	25 µl	25 µl
T7 RNA Polymerase	1 µl	1 µl	1 µl
RNase Inhibitor, Murine	1 µl	1 µl	1 µl
DHFR-His control template (125 ng/ µl)	--	2 µl	--
Linear DNA template (>100ng/µL)	--	--	250 ng
NEBExpress GamS Nuclease Inhibitor	--	--	1 µl
Water	11 µl	9 µl	to 50 µl

1. During the experimental setup, it is recommended to add the linear DNA template in the last step to allow GamS to bind and inhibit RecBCD exonuclease before RecBCD has a chance to act on the DNA.
2. Optimal GamS concentration must be determined empirically for a particular target.

4

Incubate reactions at **37 °C** , with shaking, for **02:00:00** – **04:00:00** .

Additional incubation time (maximum 10 hours) at 37°C may increase yield.

5

Analyze by method of choice or freeze at **-20 °C** for later use.