AVIDITY

AvidExpress™ Cell-Free Translation System

(Cat. # CFT-100)

Description:

Translation initiation in mammalian cells is a complex process that requires a set of specific eukaryotic initiation factors for positioning 40S ribosomal subunit at the specific translation site. Failure of this process results in aberrant initiation, premature termination or miscoding during protein synthesis. The newly synthesized proteins also require eukaryotic chaperons and membranous structure for correct folding. Contrary to bacterial and plant derived cell-free lysates, our AviExpress™ cell-free translation system (CFT) provides human HeLa S3 cell-derived natural ingredients such as translation factors, amino acids, chaperons and membranes for translation of cellular or *in vitro* synthesized mRNAs.

Features:

1. Human cell lysate-based translation system
2. The method is simple: just add supplied components and your RNA template.
3. Just as with natural mRNAs, *in vitro* transcribed RNAs containing internal ribosome entry site (IRES) or cap structure at the 5' end and poly(A) tail at the 3'end can be efficiently translated.
4. The translation products can be easily labeled with [^{35}S]Methionine or biotinylated amino acids or use our AviTag sequence in your mRNA for detection of translated protein.
5. Lysates can be used to study protein-protein, RNA-protein and DNA-protein interactions by UV cross-linking, electrophoresis mobility shift assay (EMSA) and immunoprecipitation assays.
6. The system can be scaled-up easily.
7. Biotinylated proteins can be synthesized for direct immobilization on solid surfaces coated with streptavidin or its molecular analogues

Components supplied:

Components	Cat. #	Amount	No. of tubes	Cap color
LYSATE-S	L-100	110 ul	2	green
LYSATE-IF	L-110	50 ul	2	orange
10XReaction Buffer	R-120	50 ul	1	yellow
Positive Control RNA	RNA-PC1	10 ul (1 ug/ul)	1	lavender
Nuclease-free H_2O	-	1 ml	1	blue

Storage conditions:

Store at -70 C. Avoid freeze-thaw cycle. Unused materials can be freeze-thaw once or twice without compromising much of the activities.

10X Reaction Buffer: 155 mM HEPES-KOH, pH 7.4, 10 mM ATP, 2.5 mM GTP, 300 mM phosphocreatine , 4 mg/ml creatine phosphokinase and 600 mM potassium acetate.

Materials not supplied:

Luciferase assay and protein labeling kits

Quality Control:

The HeLa S3 culture was tested negative for mycoplasma contamination. The lysates are prepared free-of detergents and tested for translation efficiencies meeting our defined criteria.

Protocol:

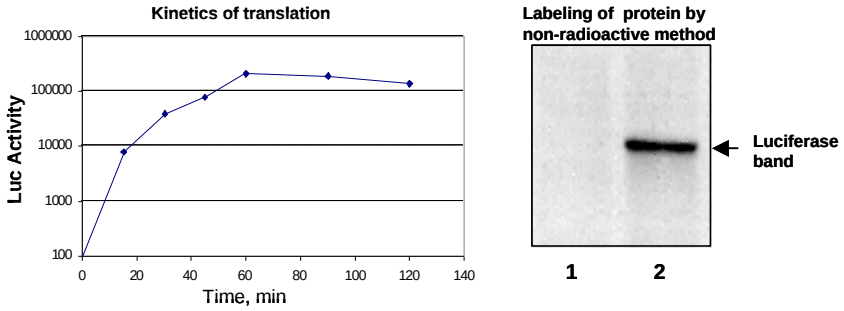
1. Add components in the following order:

LYSATE-S =	**20 ul**
LYSATE-IF =	**8 ul**
10X Reaction Buffer =	**4 ul**
mRNA =	**1-5 ug**
H_2O to	**35 to 40 ul***

**Efficient translation depends on many factors such as mRNA concentration and its stability as well as salt concentrations. The final volume can be adjusted between 35 ul to 45 ul for different templates. The reaction is assembled in the order shown here on ice. Use gloves and take precautions to avoid nuclease contamination. Addition of 1-2 ul RNase inhibitor (not supplied) is strongly recommended for efficient translation of most mRNAs, although it may not be necessary depending on the stability of the RNA template.*

A larger reaction can be carried out provided that the components are added in the same ratio For a positive control, prepare a similar reaction but with the supplied positive control RNA. For labeling of the protein, add 1-2 ul of a desired labeling material such as [^{35}S]Methionine or biotinylated amino acid-charged tRNA (e.g. Transcend System®, Promega) in the reaction.

2. Incubate the lysates at 30 ^{0}C for 1-2 hr
3. Analyze or assay for protein synthesis. The positive control contains an RNA that encodes firefly luciferase, and can be assayed for enzymatic activity or can be labeled to visualize approximately 60 kDa band.



Figures. Kinetics of an IRES-contrlolled translation in AvidExpress CFT (graph). LUC, firefly luciferase. Right panel, SDS-PAGE of translated protein followed by detection of protein band with chemiluminescence method. One ul of the above reaction was analyzed on the gel. Lane 1, control lysate without exogenous RNA; lane 2, in vitro transcribed RNA expressing firefly luciferase.

Reference:

Barton, D. J., B. J. Morasco, and J. B. Flanegan. 1996. Assays for poliovirus polymerase, 3D(Pol), and authentic RNA replication in HeLa S10 extracts. Methods Enzymol. **275:**35-57.