Wheat Germ Extract

INSTRUCTIONS FOR USE OF PRODUCTS L4380 AND L4330.

Quick PROTOCOL

In vitro Translation Protocol

Before You Begin

Remove the reagents from storage at –70°C and slowly thaw on ice.

Translation Procedure

1. Assemble the reaction components, appropriate for the label being used, in a 0.5ml polypropylene microcentrifuge tube. After adding all components, gently mix the extract by pipetting and stirring the reaction with the pipette tip. If necessary, centrifuge briefly to return the sample to the bottom of the tube. Store remaining Wheat Germ Extract at –70°C.

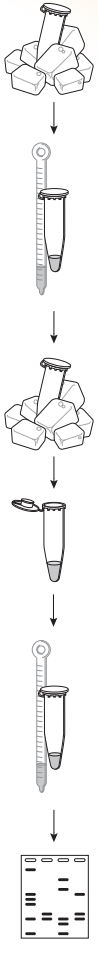
Component	Standard Reaction Using [^{35}S]methionine
Wheat Germ Extract	25µl
Amino Acid Mixture, Minus Methionine, 1mM	4µl
RNA substrate in Nuclease-Free Water (see Note 1) (10µl of control RNA [10µg])	10µl
Potassium Acetate, 1M (see Note 3)	0–7µl
RNasin® Ribonuclease Inhibitor (40u/µl) (see Note 6)	1µl
[^{35}S]methionine	2.5µl
Nuclease-Free Water to a final volume of	50µl

See Notes on back.

Small-scale reactions may be performed by reducing volumes proportionally.

2. Incubate the translation reaction at 25°C for 60–120 minutes.
3. Analyze the results of translation. Procedures are provided for determining percent incorporation of radioactive label and denaturing gel analysis of translation products.*

*See additional protocol information in Technical Manual #TM230, available online at: **www.promega.com**



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Notes

1. An unfractionated cytoplasmic RNA preparation is 90–95% rRNA and, as a result, translates poorly. Usually such preparations yield no better than 20–30% of the maximum incorporation attainable, and final concentrations of 0.1–0.2mg/ml RNA are needed to stimulate translation. Viral RNAs and poly(A)+ mRNAs (including mRNA transcribed in vitro) should be titrated to determine the optimal RNA template concentration, which will vary with the RNA template. We recommend a final concentration of 0.1–0.2mg/ml.
2. The optimal RNA concentration for translation should be determined prior to performing definitive experiments. In determining the optimal concentration, serially dilute the RNA template first and then add the same volume of RNA to each reaction to ensure that other variables are kept constant. Also see Note 1 above.
3. Optimum potassium concentration varies from 50–200mM, depending on the mRNA used. If this concentration of potassium results in poor translation of your sample mRNA, potassium levels should be reduced or increased to an optimum concentration. Certain mRNAs may also require altered magnesium concentrations. The optimum magnesium concentration for the majority of mRNAs is expected to fall in the range of 2–5mM.
4. Avoid adding calcium to the translation reaction. Calcium may reactivate the micrococcal nuclease used to destroy endogenous mRNA in the extract and result in degradation of the mRNA template.
5. Residual ethanol should be removed from mRNA preparations and labeled amino acids before they are added to the translation reaction.
6. Some template RNAs may require denaturation prior to translation. Incubate the RNA template at 67°C for 10 minutes, and immediately cool on ice. This increases the efficiency of translation, especially of GC-rich mRNA, by destroying local regions of secondary structure.
7. The addition of RNasin® Ribonuclease Inhibitor to the translation reaction is recommended but not required. RNasin® Ribonuclease Inhibitor acts to inhibit degradation of sample mRNAs by contaminating RNase.

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