ReCet™ HEK293T

A cell-free translation system obtained from Human Embryonic Kidney (HEK) 293T cells

Product	Catalog no	Rxns.
ReCet HEK293T	RC30-HEK293	30

Shipping: Dry ice

Storage Conditions: store components according to this manual

Shelf Life: 12 months

Description: ReCet™ HEK293T is a tripartite cell-free translation system that separates the key components of a translation reaction (purified ribosomes, ribosome-depleted cytoplasm, and RNA) from HEK293T cells. This allows researchers to flexibly combine and study these components in a controlled setting. The system's core components are purified ribosomes, a cytoplasmic extract, and a single exogenous control mRNA. Protein translation can be detected within 1 hour using methods like luminescence (for a luciferase-encoding mRNA) or Western blot analysis.

For Research Use Only. Not Intended for Diagnostic or Therapeutic Use.

Kit storage info

	Qty.	Storage
-20°C components	1 box	-20°C
-80°C components	1 box	-80°C

Table 1. *Kit composition (in boxes and bag) and storage temperature.*

Additionally Required Materials

- RNase-free microcentrifuge tubes
- RNase-free pipette tips
- Nuclease Free Water
- Microcentrifuge and non-stick RNase-free microfuge tubes (0.2 mL)
- Thermocycler or incubator capable of maintaining precise temperature control.
- If luminescence detection:
    - Luminometer or multimode plate reader with compatible software
    - Luciferase assay system (e.g. Promega ONE-Glo(TM) Luciferase Assay System, #E6110)
    - Luminometer compatible 96-well plate
- If Western Blot detection:
    - Gel electrophoresis full system including an electrophoresis tank, power supply, and Western Blot accessories for protein separation and visualization.

INTRODUCTION

Benefits of ReCet HEK293

- **Authentic Human System:** ReCet™ HEK293 uses functional human translational machinery, allowing you to study protein synthesis in a more physiologically relevant context.
- **Maximum Flexibility:** Unlike other systems, ReCet™ is a tripartite solution, providing ribosomes, cytoplasmic extract, and target RNA as separate components. This gives you the flexibility to mix and match as needed for your specific research.
- **Robust Performance:** Get stable protein signal. This system is particularly effective for expressing cell-type-specific proteins that are often difficult to produce in other systems, such as rabbit reticulocyte lysates.
- **Scalable:** The system is easily customizable for high-throughput screening or can be adapted with ReCet™ components derived from different cell types (for custom ReCet kit please contact us at orders@immaginabiotech.com).

Better Than Traditional Methods:

- Accurate translation delivers full-length protein compatible with downstream applications
- Protein translation is optimized with 5'cap mRNA to cover most of the RNA drug discovery processes. IRES-based RNA can be translated as well but less efficiently, according to the cell-specific physiological mechanisms (Fig. 1).
- Capable of expressing proteins with cell-type specific post-translational modifications

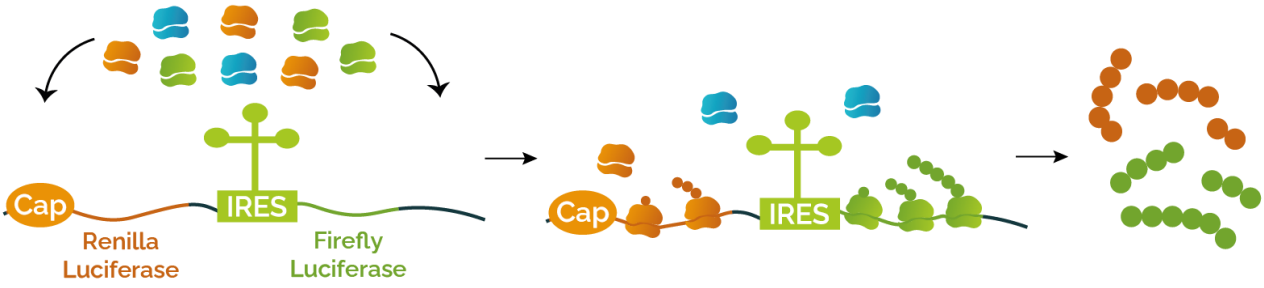

Fig. 1 ReCet™ 5'cap and IRES mRNA translation

Applications:

- Identification and validation of translatome-specific small molecule inhibitors or novel translation inhibitors
- Screening mRNA drugs translation efficiency
- Screening mRNA drugs *cis-elements* (e.g. UTRs) in a cell-type specific setting
- Screening of RNA targeted small molecules to modify translation

- Ribotoxic Stress Response: understand how stress triggers the ribotoxic stress response and to identify the sensitive molecules within the translational complex
- Assessing *specialized* translation
- Express cytotoxic proteins
- Express proteins to measure enzyme activity

This cell-free system is <u>not</u> optimized for scaling up protein production *in vitro*.

TECHNICAL OVERVIEW

ReCet™ HEK293T can be used to resolve which aspects of the translational machinery are selectively affected by conditions that trigger changes in ribosome activity and protein production. The kit contains all the cellular components required for protein synthesis, including ribosomes, initiation factors, elongation factors and tRNA (Fig. 2).

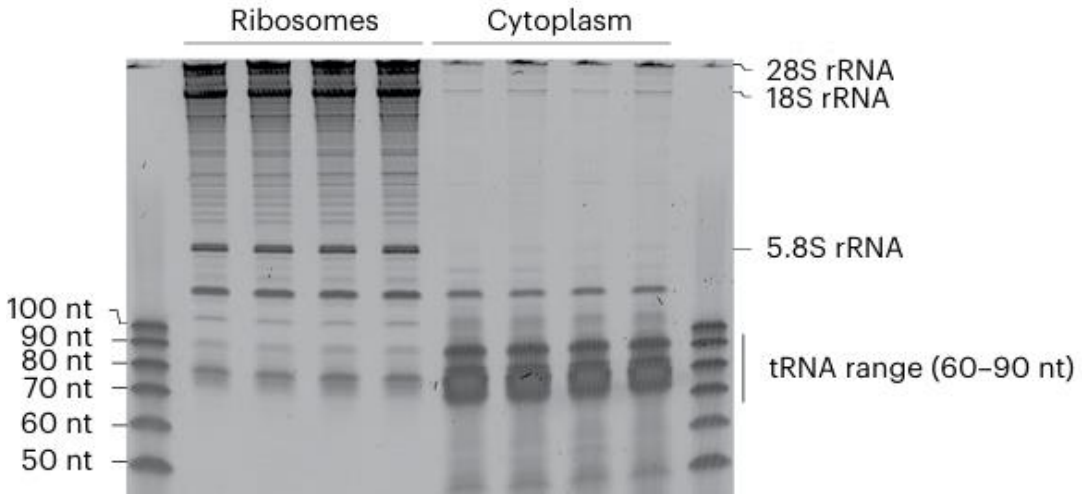

Fig. 2 Total RNA isolated from either ribosome or cytoplasmic ReCet components was separated on 6% (wt/vol) urea agarose gel and stained with SYBR-gold. A vast majority of tRNAs fractionate with the cytoplasm, whereas the rRNAs 28S, 18S and 5.8S are found exclusively in the ribosome fraction. Figure adapted from (1) Arendrup et al., 2025.

When supplemented with dedicated buffers, salts, ATP mix and an RNA template, this system can synthesize protein for up to several hours with a canonical logarithmic curve that reaches its maximum efficiency per unit of time after 45 min-60 min of incubation (Fig. 3).

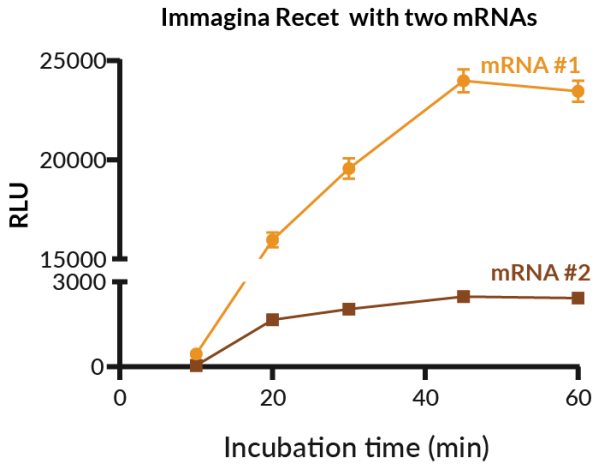

Fig. 3 In vitro luciferase expression was performed using the ReCet HEK293T system with two distinct luciferase mRNAs having a different cap and nucleotide sequence. Samples were analyzed in a 96-well plate by measuring luminescence at indicated time points. The plate was maintained at 25 °C during the readings. Luciferase activity correlate to µg/mL of active protein. Luminescence is normalized over negative control (without luciferase mRNA).

A reference mRNA (5'-cap Luciferase transcripts at a concentration of 150 ng/µL stock solution) is included in the kit as positive control for reference in downstream applications.

With ReCet™, the cytoplasmic extract, ribosome complexes, and mRNAs can be mixed from different sources and flexibly combined. Each component can be kept constant or independently varied and subjected to specific treatments before reconstitution, enabling precise dissection of their contributions to translation (Fig. 4).

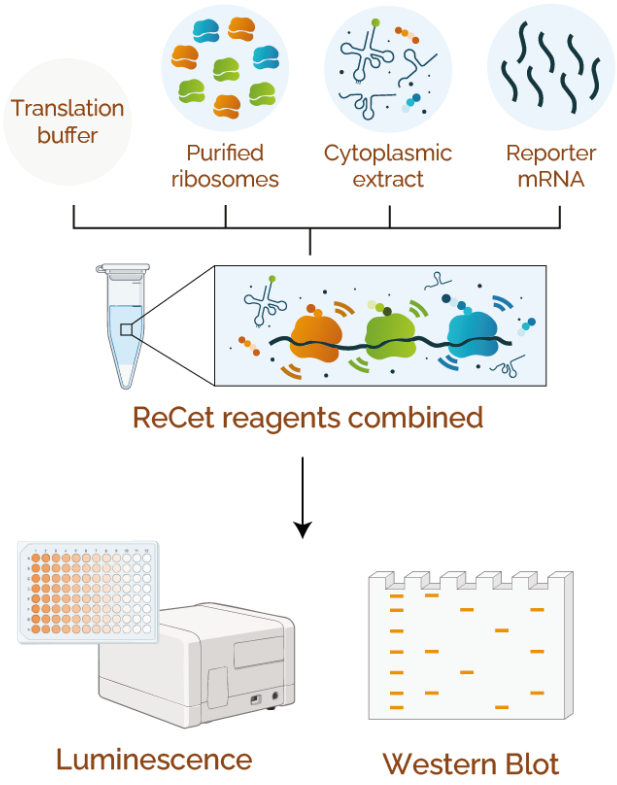

Fig. 4 ReCet™ workflow

This system can be combined with other on-the-shelf or custom ReCet tripartite cell-free translation systems from different cell types or conditions. These can be ordered by contacting Immagina Biotechnology at orders@immaginabiotech.com.

PROTOCOL OVERVIEW

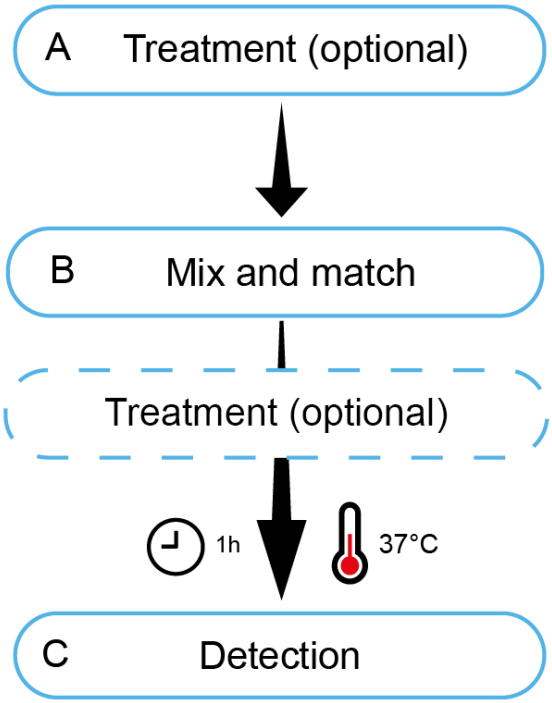

Fig. 5 ReCet™ Protocol workflow

RECOMMENDATIONS

- Store all components at the suggested temperature. If stored and handled properly, reagents are stable for at least 12 months.
- RNase contamination will compromise results**.** Always wear gloves, work in a clean, dust-free environment, and use only RNase-free tips and microcentrifuge tubes.
- Prior to start the experiment please retrieve the Translation Buffer (10X), Ribosome Extract, and Cytoplasmic Extract from –80 °C storage and **thaw slowly on ice for 20–30 minutes.** Do not thaw by hand or use any heat source, as rapid thawing may compromise performance. Upon the first thaw, aliquot any unused portion immediately and return it to –80 °C as soon as possible. **Multiple freeze–thaw cycles may adversely affect activity and performance.**
- For troubleshooting, please check appendixes.

SPECIFICATIONS

- **Origin cells:** HEK293T
- **RNA Templates and Positive control**. mRNA Luciferase (cat. no. #IBT0621) is include in the kit. The sequence of the transcript can be provided to clients upon request.

A. TREATMENT

ReCet_HEK293 components needed in this section:

Kit component	Cat. nr.	Volume	Storage	Type	Vial cap color
Translation Buffer (10X) (TB)	#IBT0611	100 ul	-80°C	vial	
Cytoplasmic extract HEK293 (CE_{HEK})	#IBT0621	3 x 60 ul	-80°C	vial	
Ribosome extract HEK293 (RE_{HEK})	#IBT0631	3 x 13 ul	-80°C	vial	
Cap-mRNA Firefly Luciferase (FLuc)	#IBT0363	40 ul	-80°C	vial	

Treatments can be applied to individual components (ribosomes extract, cytoplasmic extract, or mRNA reporter) before assembling them or to the fully reconstituted translation system (See Appendix 1). Please note that, when comparing different reactions in your experimental setup, you need to keep the volume of ReCet HEK293 reactions consistent. Therefore, if you use a water/buffer to dissolve a drug, you must add an equal volume of that same water/buffer to your control reactions. Examples of treatment with translational inhibitors can be found in the Appendix 1A.

B. MIX AND MATCH

ReCet_HEK293 components and needed in this section:

Kit component	Cat. nr.	Volume	Storage	Type	Vial cap color
Translation Buffer, 10X (TB)	#IBT0611	100 µl	-80°C	vial	
Cytoplasmic extract HEK293 (CE_{HEK})	#IBT0621	3 x 60 µl	-80°C	vial	
Ribosome extract HEK293 (RE_{HEK})	#IBT0631	3 x 13 µl	-80°C	vial	
Cap-mRNA Firefly Luciferase, 150 ng/µL(FLuc)	#IBT0363	40 µl	-80°C	vial	
RNase Inhibitor, 100X (RNI)	#IBT0641	15 µl	-20°C	vial	
Saline solution 1 (Sal1)	#IBT0651	30 µl	-20°C	vial	
Saline solution 2 (Sal2)	#IBT0652	30 µl	-20°C	vial	
ATP	#IBT0174	10 µl	-20°C	vial	

☐ **1.1** Thaw on ice all the ReCet HEK293 components (please read Recommendations before!)

☐ **1.2** Mix the following reagents on ice, following the order reported in the table, in a 0.2 mL nuclease-free PCR tube. The volumes reported in the table are calculated for 10 reactions (including 10% extra volume). Scale up or down according to your experimental needs. **NOTE**: all solutions provided by Immagina are sufficient to prepare 3 times the "master mix" below, which should allow you to process ten samples. All unused portions of both cytoplasmic and ribosome extracts must be returned to –80°C immediately (see Recommendations, page 8) to avoid repeated freeze-thaw cycles.

Component	**Volume (µL)**
Translation Buffer (10X) (TB)	11
RNase Inhibitor (RNI)	1.1
Saline solution 1 (Sal1)	2.9
Saline solution 2 (Sal2)	2.7
Cytoplasmic extract (CE_{HEK})	55
ATP	1.5
Ribosomes extract (RE_{HEK})	11
Nuclease free water (up to 110 µL)	13.8
FLuc mRNA Reporter *	11
Total volume	**110 µL**

**for Negative control: Replace FLuc mRNA Reporter volume with nuclease-free water to keep the total volume constant.*

☐ **1.3** Aliquot 10 µL of the mix in a 96-well plate or in 0.2 mL nuclease-free PCR tubes and incubate the reaction at 37 °C in a thermocycler for 1 hour (the incubation time can be extended up to 3 hours).

☐ **1.4** The reaction can be used immediately for protein detection or stored at 4 °C for up to 1 day, or at ≤ –20°C for longer periods.

C. DETECTION

The luciferase translation output can be measured by immunoblotting or with a luciferase assay after incubation with a Luciferase Assay System for 45 minutes at 22-25 °C. See Paragraph C3 for alternative detection methods.

A. Luciferase assay

We recommend using a FLuc detection system with moderate signal strength and half-life, which is well-suited for high- or ultrahigh-throughput applications. Assay kits such as the ONE-Glo™ Luciferase Assay System (Promega #E6110) or equivalent are appropriate. For detection, transfer 10 µL of the reaction mix into a 96-well plate, dilute with 40 µL of PBS, and measure the resulting luminescence to quantify protein production. Discard any remaining bulk reaction mixture.

B. Immunoblotting

Load all the 10 µL reaction in the gel with the appropriate protein loading dye. The molecular weight of Firefly luciferase included in the kit is approximately 62 kDa. Please note that other luciferase variants, such as Renilla or Gaussia, could have different molecular weights. A high-quality anti-luciferase primary antibody is essential. These are available from various commercial suppliers (e.g., Promega, Thermo Fisher Scientific, Cell Signaling Technology, Abcam). You may also need a secondary antibody that is specific to the host species of your primary antibody (e.g., goat anti-rabbit IgG if your primary antibody is a rabbit monoclonal/polyclonal).

As controls, it is possible to load on the gel:

- **Positive Control**: A ReCet reaction expressing FLuc mRNA Reporter.
- **Negative Control**: A ReCet reaction without FLuc Reporter (substitute the volume of FLuc mRNA Reporter with water)
- **Loading Control**: An antibody against a housekeeping protein (e.g., RPL6, RPL10A, RPS18) can be used to ensure equal protein loading across samples.

C Alternative detection methods

- The FLuc RNA control can be substituted with a **Green Fluorescent Protein (GFP)** RNA. The expressed GFP can be visualized and quantified through several methods, including immunoblotting, radioactive labeling, or direct detection of fluorescence. For a rapid visual assessment, place the GFP reaction tubes directly under a microscope or imaging equipment equipped with a FITC filter (excitation/emission: 482/512 nm). Alternatively, a small volume (1-2 µL) can be spotted onto laboratory film and visualized with a fluorescent imaging system. When using a 96-well plate format and dedicated plate readers, successful detection depends on the sensitivity of the instrument and the quantity of protein produced per reaction.
- **Radiolabeling**: as described in (1) Arendrup, et al. (2025), newly synthesized proteins can be detected by adding [35S]-methionine to the reaction, followed by protein gel electrophoresis and autoradiography.

- **Biotinylation**: The cell-free translation reaction can be performed with Transcend™ tRNA (Promega, L5061), a precharged and biotin-labeled lysine tRNA, for detecting products from complete poly(A)+ mRNA pools.

APPENDIX 1: EXAMPLES OF TREATMENTS

A Translation inhibitors treatments: CHX treatment and Puromycin

CHX was introduced at 0.1 µg/µL and 1 µg/µL 5 minutes after step 1.2 of the MIX AND MATCH section. The translation reaction was subsequently incubated at 37 °C for 55 minutes. The resulting histogram depicts the dose-dependent reduction of protein synthesis caused by CHX (Fig.6A) .

A similar experiment was conducted with puromycin. Puromycin was added at 3 different concentrations immediately after step 1.2 of the MIX AND MATCH section and the translation reaction was incubated at 37 °C for 1h. At nanomolar concentrations, puromycin resulted in the complete inactivation of the translation system (Fig. 6B).

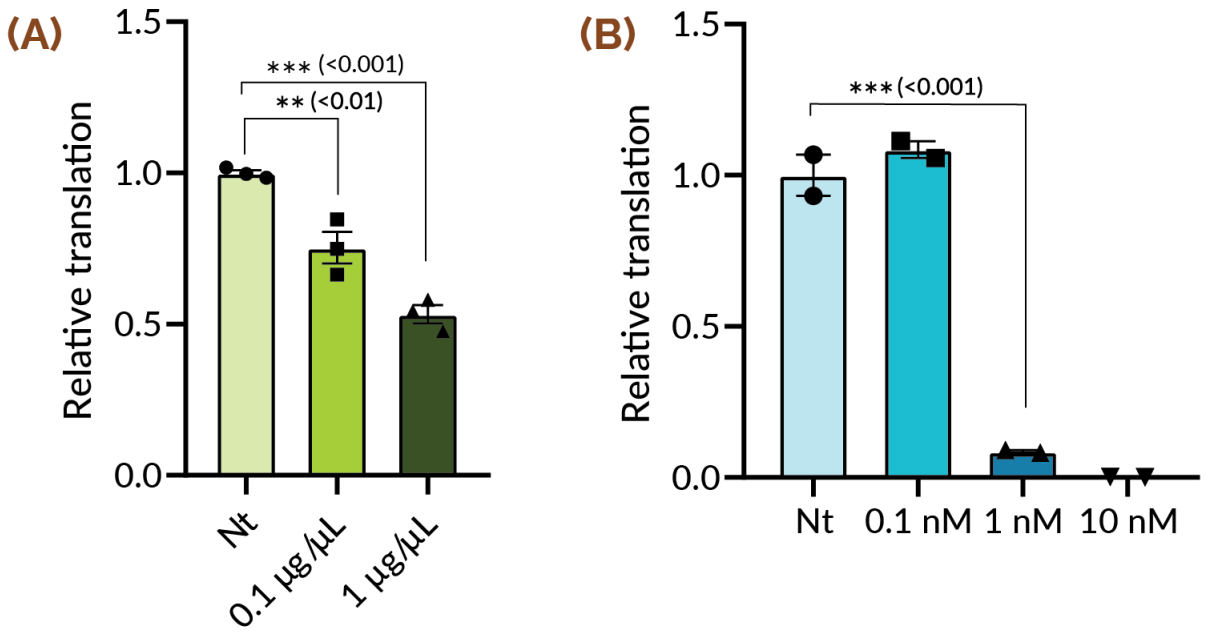

Fig. 6 Effect of cycloheximide (A) and puromycin (B), two well-known translation inhibitors. The results demonstrate a clear dose-dependent reduction in protein synthesis, highlighting ReCet's precision and reliability for drug-testing applications. Data are mean ± SD statistical test: one-way ANOVA.

B MNase treatment:

The ReCet™ kit can be used after depletion of endogenous mRNAs by treating the Ribosome extract (catalog number #IBT0631) with MNase (Fig. 7). The MNase treatment, while suitable for any volume of purified ribosomes, is more effective with larger volumes like 100 µL, as it minimizes dilution effects on translational output; adding a larger volume of ribosomes to the final reaction can

compensate for any necessary dilution. To perform the treatment, follow the protocol reported in Box 2 (Pag. 7) of (1) Arendrup, et al. (2025). Please note that MNase treatment may reduce ReCet™ efficiency.

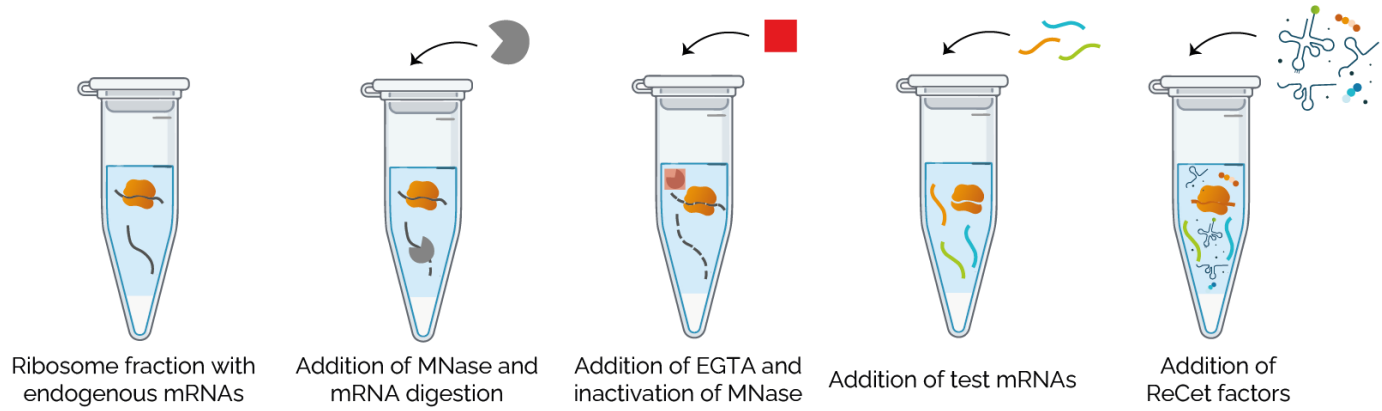

Fig. 7 Overview of the depletion of endogenous mRNAs by treating the ReCet Ribosome extract with MNase.

C Alternative approaches

Other flexible options to investigate translation are reported on the following key references:

- (2) Snieckute *et al.* 2023
- (3) Ryder *et al.* 2023

References:

(1) Arendrup, et al. Nature Protocols (2025). https://doi.org/10.1038/s41596-025-01155-7
(2) Snieckute, G. et al. Science 382, eadf3208 (2023): https://doi.org/10.1126/science.adf3208
(3) Ryder, L. et al. Cell Death Dis. 14, 467 (2023): https://doi.org/10.1038/s41419-023-05997-5

APPENDIX 2: TROUBLESHOOTING

1. Luminescence not detected in positive control reaction

Verify that the correct setup is used for your experiment in the plate reader and test the activity of your luciferase reaction assay. Furthermore, proper storage of the ReCet components is critical for their activity. Unused ReCet™ reactions should be stored in nuclease-free tubes at -80°C, and to prevent a loss of activity, you should not exceed two freeze-thaw cycles. Please note that DMSO is inhibiting the ReCet™ reactions.

2. No expression of target protein

The most common issues are related to sample storage. Ensure all reagents are stored at a proper temperature of -80°C and are not subjected to many freeze-thaw cycles. RNA quality is also critical, so it's recommended to remove truncated mRNA or any inhibitors or salts. Furthermore, maintaining an RNase-free environment is essential to prevent mRNA degradation. This can be achieved by using gloves and sterile, RNase-free labware. Finally, if the protein of interest is sensitive to proteases, adding protease inhibitors like Aprotinin or Leupeptin to the lysate can prevent protein degradation. Please note that DMSO - used to dissolve compounds not included in the kit - is inhibiting the ReCet™ reactions.

3. Smaller band size than predicted

Ensure that your genes of interest do not contain any premature stop codons within the open reading frame. The presence of a stop codon will prematurely terminate translation, resulting in a truncated and often non-functional protein. It is essential to verify the sequence of your cloned genes to avoid this issue. Additionally, please verify the specificity of your antibody

4. Low protein yield

To ensure optimal results and protein expression, it is crucial to follow the correct protocol. First, confirm that all translation reactions are performed at the recommended incubation temperature of 37°C. Additionally, the order of reagent addition is critical. Please note that DMSO - used to dissolve compounds not included in the kit- is inhibiting the ReCet™ reactions.

To address low protein yield, consider increasing the reaction scale combining multiple reactions (up to 30 µL reaction volume) to ensure sufficient product for downstream analysis. If the cell model proves unsuitable for your protein of interest, you may need to switch to a different ReCet model derived from a more compatible cell type (contact us at orders@immaginabiotech.com for more information). Furthermore, to exclude issues with an unstable RNA template, it is essential to check its integrity using a Agilent TapeStation OR Bioanalyzer System and to ensure all reagents and equipment are RNase-free.

Contacts

Info

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Notes: