interchim

Genelator™
in vitro Transcription & Translation Kit

INSTRUCTION MANUAL

Catalog No. 12011 mini scale

For Research Use Only

F-Detector™
Cell Free Labeling Kit

INSTRUCTION MANUAL

Catalog No. 12021 mini scale

For Research Use Only

iNtRON BIOTECHNOLOGY
iNtRON Biotechnology, Inc.
TEL : +82 31 778 7890 FAX : +82 31 736 7245
www.intron-innoplex.com | www.intronbio.com

ORDER INFORMATION

Please include the following information when placing your order.

1) Institution or customer account number
2) Shipping address
3) Billing address
4) Purchase order number
5) Name of end user
6) Telephone of end user
7) Name of purchasing agent
8) Quantity, catalog number, and the description of the product
9) For Visa or MasterCard orders, please provide card type, name on the card, account number, and expiration date

Order by Telephone (+82)505-550-5600, WR or 0505-550-5600, KR
Order by FAX (+82)505-550-5660, WR or 0505-550-5660, KR
Order by E-mail intronbio@intronbio.com info@intronbio.com

PRODUCT INFORMATION

❑ PRODUCT LIMITATION

All iNtRON products are "For research use only and are not intended for diagnostic, therapeutic or any *in vivo* use in Human subjects".

❑ TECHNICAL SERVICES

iNtRON offers a full range of technical services that include a wide range of analytical capabilities and services. In addition to our analytical services, we offer application testing for different molecular biology, cell culture, clinical, and diagnostic reagents.

❑ QUALITY GUARANTEE

iNtRON provides the scientific community with professional-quality laboratory products. If you are not satisfied with a iNtRON product, write to iNtRON Customer Service Depart., 358-11, Sangdaewon-Dong, Joongwon-Ku, Kyungki-Do, 462-120, KOREA.

❑ CUSTOM SERVICES

If you have any questions for our products, please contact us.

TEL (+82)505-550-5600, WR or 0505-550-5600, KR
E-mail intronbio@intronbio.com

You can meet a colorful manual (pdf file) in our website.

MATERIAL PROVIDED

For Laboratory Use. The Genelator™ kit contains sufficient reagents to perform approximately 25 times × 45μl translation reactions. Includes :

Material Provided	Quantity
(1) Genelator™ Solution I	375 μl × 1 ea.
(2) Genelator™ Solution II	465 μl × 1 ea.
(3) RNase-Free dH_2O	1ml × 1 ea.
(4) Positive Control DNA (EGFP)	25 μl (200ng/μl) × 1 ea.
(5) Handbook Guide	Protocol

You make Genelator™ master mix by mixing Solution I and II before use (refer to protocol).

INTELLECTUAL PROPERTIES

This **Genelator™** has been co-developed with DreamBiogen, Co. (www.dreambiogen.com). Furthermore, Materials in this application and Methods of use are covered by various intellectual Properties.

Trademarks

Genelator™, F-Detector™, DNA-spin™, DNA-midi™, MEGA-spin™, PCRquick-spin™

Patent & Patent Pending

KR10-24695	KR10-131166	KR10-401296	KR10-399337	US5,593,856
KR10-009628	PCT/KR2004/000302		JP2001-47494	JP2001-57514
US09/783,721	US09/792,647	EP01420032.3	EP01420050.0	

STORAGE and STABILITY

The **Genelator™ *in vitro* Transcription/Translation Kit** is shipped on dry ice. All components must be **stored at below –70 ℃**. Do not refreeze and thaw more than five times. These temperature fluctuations can greatly alter product stability. When stored under the above conditions and handled correctly, both kits can be kept for 12 months without showing any reduction in kit performance.

PRODUCT USE LIMITATION

The Genelator™ kit is developed, designed, and sold for research purposes only. It is not to be used for human diagnostic or drug purposes or to be administered to humans. All due care and attention should be exercised in the handling of many of the materials described in this text.

The Human Genome Project has led to a greater understanding of human genes. Following complete sequencing of the human genome, we are to about to enter a post-genome era in which the function and structure of proteins encoded by the sequenced genes need to be studied. Therefore, great efforts have been made to develop technologies for the production of proteins using recombinant technology. Using modern protein engineering methods, which include cloning of DNA sequences and the *in vivo* expression of genes, it is possible to produce specific proteins in large amounts and also to produce proteins with improved or altered biological activities.

Several factors must be carefully considered when producing recombinant proteins using *in vivo* expression methods. Cells must be transformed with an expression construct (plasmid/vector DNA), and transformants containing the correct construct must be selected and cultivated. However, the **Cell-Based Direct Protein Expression** method has some problems. Firstly, the protein to be used in analysis should be produced in a soluble form. The most common technique for Cell-Based Direct Protein Expression involves using a microorganism such as *E. coli*. The majority of proteins expressed by this *in vivo* expression method are, however, produced in an insoluble or aggregate form such as inclusion bodies.

Although solubilization/refolding procedures are known to transform these proteins into soluble proteins, these are very time-consuming and often inefficient processes, and also they are not generally applicable to all proteins. It is thus most desirable to obtain soluble proteins directly without the requirement for refolding. The production of inactive, insoluble proteins during heterologous cell-based expression results from the fact that, owing to the difference between the rate of protein synthesis and the rate of protein folding, interactions occur between hydrophobic residues exposed from the folding intermediate of the protein of interest, thereby aggregates are formed.

In order to obtain soluble proteins in cell-based expression systems, many methods are utilized, for example, protein engineering approaches such as substitution of original amino acids with a different amino acids to improve solubility of proteins; fermentational approaches such as temperature adjustment, pH adjustment and/or addition of additives; fusing approaches whereby the protein of interest is fused to proteins of high solubility; and co-expression approaches whereby foldases such as DsbA or PPIase are simultaneously expressed with the desired protein. In addition, there is another technique involving co-expression with a chaperone family protein such as GroEL/GroES or DnaK/DnaJ/GrpE for the production of the soluble protein of interest. Molecular chaperones used in Genelator™ System are a set of proteins which participate in protein folding and prevent the aggregation of newly synthesized proteins and lead to the correctly folded protein.

In Cell-Based Direct Protein Expression, overexpression of proteins that are toxic to the host cells can be difficult. Cell lysis and procedures used for purification of

protein from whole cell lysates can be complicated; problems may arise because of aggregation or degradation of proteins within the cell. In most cases these limitations can be overcome by the use of Cell-Free Protein Synthesis Systems which are a very attractive alternative to classical *in vivo* expression systems.

Cell-Free Protein Synthesis (also termed *in vitro* transcription/translation system) has been a standard tool for the rapid characterization of gene products of interest for almost two decades. The use of cell-free systems for the *in vitro* expression of proteins continues to rapidly expand with various applications in basic research, molecular diagnostics and high-throughput target/drug discovery. Increasingly, extract-based expression tools are being used in functional genomic applications as a “bridge” between traditional genomic and proteomic approaches. Research is moving toward the systematic characterization of gene expression and protein function. *In vitro* expression technologies offer significant time-savings over cellular and whole animal approaches and are generally easy to perform. High-throughput protein-expression screening based on cell-free protein synthesis is useful for large-scale proteomic efforts aimed at identifying and characterizing expressed proteins.

In vitro transcription and translation generates proteins by coupled or successive transcription and translation in cell-free extracts of prokaryotic or eukaryotic cells. The advantages of *in vitro* transcription/translation systems include significant time savings, the possibility to produce proteins that are toxic or contain modified or isotope-labeled amino acids, a high protein yield per unit volume, and the ability to adapt reaction conditions to the requirements of the synthesized protein (e.g., the inclusion of protease inhibitors). Proteins produced by *in vitro* trasnscription/translation can be used for a wide variety of downstream applications, including activity assays, structural and mutational analyses, protein–protein interaction studies, and the expression and analysis of open-reading frames. iNtRON

Many proteins are inherently poorly expressed, insoluble, cytotoxic, or susceptible to proteolysis, any of which can result in low-soluble *in vivo* expression yield. Although solubilization/refolding procedures are known to transform these proteins into soluble proteins, these are very time-consuming and often inefficient processes, and also they are not generally applicable to all proteins. It is thus most desirable to obtain soluble proteins directly without the requirement for refolding. For this requirement, **Cell-Free Protein Synthesis** can be considered as an alternative due to its flexibility in manipulating protein folding.

In spite of such an advantage in promoting protein folding, production of aggregation-prone proteins originated from eukaryote using conventional prokaryotic cell-free protein synthesis systems based on *E. coli* frequently results in rapid aggregation of these proteins, although to a lesser extent than in cell-based *in vivo* expression systems. This aggregation is problematic for direct functional analysis using cell-free translation mixture without purification.

The **Genelator™ *in vitro* Transcription/Translation Kit** uses highly productive *E. coli* lysates, which contain all transcriptional and translational machinery components (chaperones, ribosomes, ribosomal factors, tRNAs, aminoacyl-tRNA synthetases, etc.) as well as T7 RNA polymerase. It is a coupled transcription–translation system that can be used to express full-length proteins from T7 or *E. coli* promoters in a single-step reaction using supercoiled (plasmid) DNA templates. Using the **Genelator™** Kit, up to 600 µg/ml biologically active protein can be synthesized within 60 minutes. The synthesis reaction can be easily scaled up (to produce mg amounts of protein). The amount of protein synthesized increases linearly with increased reaction volume.

The **Genelator™ *in vitro* Transcription/Translation Kit** is more suitable for the enhanced soluble production of aggregation-prone protein compared to conventional cell-free protein synthesis. **Genelator™** Kit is prepared from genetically engineered *E. coli* strains which are modified to have enhanced levels of folding-related factors as a folding aid for better solubility of proteins. **Genelator™** Kit serves as a powerful technology for the production of aggregation-sensitive proteins originated from eukaryotes.

We expect this **Genelator™** Kit will be helpful in production a higher level of soluble proteins for further biochemical analysis and proteomics research such as structural genomics and functional genomics. And it is applicable to preparation of protein samples required in the actively growing research areas such as structural genomics, functional genomics, protein chip, diagnosis, protein engineering, bioelectronics including biosensor, and lab-on-a-chip. **iNtRON**

Genelator™ Short Procedure

Thaw quickly Solution I and II reagents

↓

Mix Solution I and II reagents
[Genelator™ Solution I + Genelator™ Solution II]

↓

Add DNA template and RNase-free water

↓

Incubate at 37°C for 60 ~ 90 minutes

↓

Separate translation products by SDS-PAGE

↓

Detect

Fig 1. Short Procedure of Genelator™ Kit

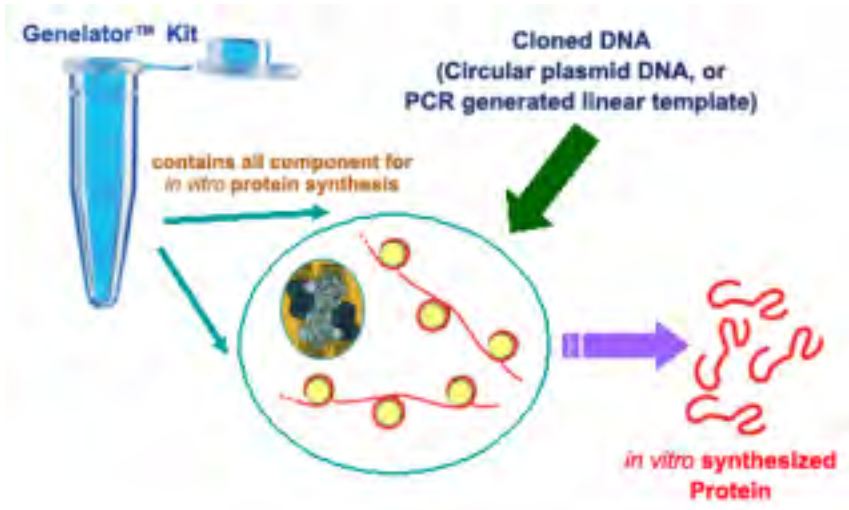


Fig 2. Schematic Showing *in vitro* Transcription/Translation of Proteins

The **Genelator™** Kit can be used to express proteins from a variety of DNA templates as long as they contain a T7 or strong *E. coli* promoter such as Lac promoter. Suitable DNA templates include supercoiled plasmids. The greatest yields of protein are obtained using template DNA of the highest purity. The high purity of plasmid DNA is very important for *in vitro* transcription/translation system. Especially, the ratio of supercoiled DNA to total plasmid DNA is significantly important. You have to choose more better kits among various commercial products.

We only guarantee the quality of iNtRON and Qiagen plasmid kit.

We recommend our **DNA-spin™** Plasmid DNA Purification Kit **(CAT. 17093)** and **DNA-midi™** Plasmid DNA Purification Kit **(CAT. 17251)** . The amount of plasmid DNA added to each 45 µl *in vitro* transcription and translation reaction should be 0.5 µg for plasmids up to 5 kb in size, or 1 µg for plasmids >5 kb. The following tips are technical information for the greatest yields of protein by using **Genelator™** *in vitro* transcription/translation kit.

Choice of Plasmid Kit

There are many commercialized plasmid DNA extraction column kits. However, we only guarantee the quality of plasmid DNA when you use our **iNtRON's DNA-spin™/DNA-midi™** kits or **Qiagen's (German)** kit.

Effect of Chemicals

If you want to extract plasmid DNA manually without using column kit, avoid adding excessive salts or glycerol when extracting DNA. Especially, do not resuspend plasmid DNA with TE (Tris/EDTA) buffer. Instead of TE buffer, you can use distilled H_2O. The EDTA inhibit *in vitro* transcription/translation reaction. The reaction activity may be inhibited by NaCl (<50mM), glycerol (<1%), or by very small amounts of Mg^{2+}, or potassium salts. Our **DNA-spin™** and **DNA-midiT™ Plasmid DNA Purification Kit** is suitable for *in vitro* transcription/ translation system. Any components in DNA-spin™ kit do not inhibit the efficacy of Genelator™ reaction.

Must proceed both washing A/B steps in preparing plasmid DNA.

Washing A and B Step

When you purify a plasmid DNA by using the column kits, you have to process two kinds of Washing Steps. Both iNtRON's DNA-spin™ kit and Qiagen's kit have two kinds of washing solutions including washing buffer A and B. Generally, the washing buffer A step is an optional step useful for removing endonuclease I (*endA*$^+$) enzyme. However, **in this *in vitro* transcription/translation step, you should use those two Washing Buffer A and B although your host *E. coli* is *endA*$^-$ (endonuclease I negative) strain** (refer to next page).

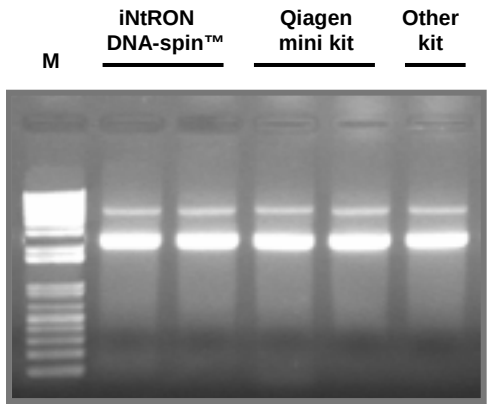
(1) EFFECT OF PLASMID DNA QUALITY

We only guarantee the quality of iNtRON and Qiagen plasmid kit.

The EGFP (Stratagene) plasmid DNA has been only used in testing our kit. We provide an original GFP plasmid DNA as a control. You may purchase and use EGFP DNA as a control.

Using various commercialized plasmid DNA extraction kits, we compared the protein synthesis yields. From the results, although the plasmid DNAs show similar yields under UV-transilluminator, the protein yields are very different (under laser-based fluorescence scanning device). So, you should be careful to choose plasmid DNA extraction kit.

We guarantee only iNtRON's DNA-spin™ kit and Qiagen's spin kit.

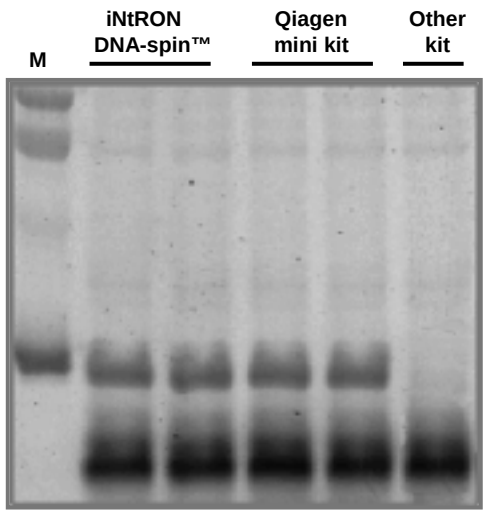


- **Plasmid DNA : pD2HM-EGFP**
- **Host *E. coli* : JM110 (*endA*⁺ strain)**

Nicked circles

Supercoiled

- **$OD_{260/280}$: 1.75 ~ 1.85**
- **Adjust to 200ng/μl (EGFP)**
- **2μl loading at 1.0% Agarose gel**



- **Genelator™ master mix : 11.2μl (15μl rxn.)**
- **EGFP plasmid DNA 2μl (200ng/μl)**
- **F-Detector™ solution : 1μl**
- **1 hr *in vitro* reaction at 37°C**
- **12% SDS-PAGE**
- **Under FLA scanner**

EGFP 29.6 KDa

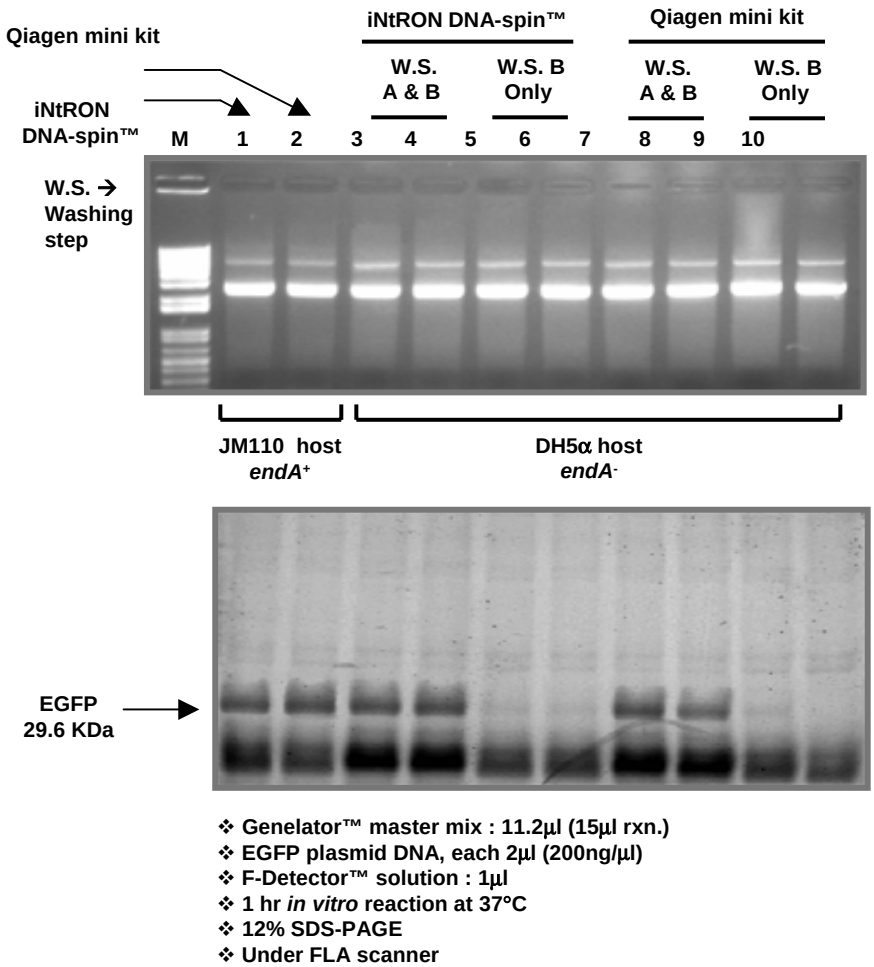
• GFP, Green fluorescence protein
• EGFP, Enhanced green fluorescence protein

(2) EFFECT OF WASHING STEP

Must proceed both washing step A and B in extracting plasmid.

We have compared the effect of Washing Steps during *in vitro* transcription/ translation reaction. We have purified two types of EGFP plasmid DNAs (DH5α host, *endA*$^-$; JM110 host, *endA*$^+$) with Washing Step A and/or B, or Washing step A/B, respectively, according to the instruction's manual. From results, you can show that the protein yields are very low when only washing step B was processed during plasmid DNA extraction in DH5α strain (*endA*$^-$).

The optional washing step (Washing Buffer B) need to get more efficient results in both **iNtRON's DNA-spin™** kit and **Qiagen's** mini kit, although the *E. coli* strains are endonulcease I negative strain (*endA*$^-$).



- ❖ **Genelator™ master mix : 11.2µl (15µl rxn.)**
- ❖ **EGFP plasmid DNA, each 2µl (200ng/µl)**
- ❖ **F-Detector™ solution : 1µl**
- ❖ **1 hr *in vitro* reaction at 37°C**
- ❖ **12% SDS-PAGE**
- ❖ **Under FLA scanner**

Prepare master mix by mixing solution I and II. Because the expiration date of master mix is short, so prepare an appropriate amount as you need.

■ The *in vitro* transcription/translation system is extremely sensitive to nuclease contamination. Always wear gloves and use RNase- and DNase-free reaction tubes and tips.

■ When you extract plasmid DNA by using iNtRON DNA-spin™ kit or Qiagen spin kit, you must proceed both washing A and B steps.

■ The vector suitable for this system is T7 promoter based or *Lac* promoter based vector system (we recommend the T7 promoter system for best protein synthesis). If you use other promoter based vector systems such as SP6 promoter, please add an suitable RNA polymerase.

■ Except for the actual transcription-translation incubation (37 ℃), all handling steps should be carried out on ice (important).

■ The Solution I and II in the **Genelator™** Kit is stable for 12 months at -70°C. However, master mix (mixed with solution I and II) has short expiration time of within 60~70 days.

■ To determine the background level of protein synthesis, always include a no-template control reaction (negative control) in your experiment. Also, to determine the success of reaction, you can perform same reaction with GFP positive control DNA (freely supplied GFP vector DNA). The qualitative GFP estimates could be made using a UV-lamp (360 nm) in a dark room.

■ The recommended incubation temperature for protein synthesis is 37 ℃, but lower incubation temperatures may improve protein solubility in some cases.

Prepare master mix by mixing solution I and II. Because the expiration date of master mix is short, so prepare an appropriate amount as you need.

Expiration Time

Solution I and II
: 12 months at -70°C
Master mix
: 2~3 months at -70°C

Reaction Volume

45µl reaction
: 33.6µl master mix
15µl reaction
: 11.2µl master mix

The **Genelator™** kit contains two kinds of Genelator™ solutions (solution I and II). You must prepare the mixture of solution I and II (**master mix**) for translating reaction. When you prepare the master mix solution, refer to table. Especially, once mixed, the expiration date of the master mix is within 60~70 days at -70 ℃, although each solution I and II are stable for 12 months at -70 ℃. So, you may prepare the master mix as you need, then aliquot and store at -70°C. The remnant solution I and II must be quickly re-frozen at -70 ℃. However, do not refreeze and thaw solution I and II more than five times. The temperature fluctuation can greatly alter the translation activity.

1. Remove the reagents (solution I and II) from storage at -70°C. Rapidly thaw Solution I and II by hand-warming and place on ice.

2. Prepare master mix solution by mixing solution I and II on ice according to the following **table 1** as much as you need. The rest of solution I and II must be quickly re-frozen at -70°C.

Table 1. Preparation for Master Mix and Aliquot List

Solution **I** (µl)	15µl	75µl	150µl	225µl	300µl	375µl
Solution **II** (µl)	18.6µl	93.0µl	186µl	279µl	372µl	465µl
Total master mix	**33.6**µl	**168**µl	**336**µl	**504**µl	**672**µl	**840**µl
Tubes (**33.6µl aliquot** for **45µl rxn**. volume)	1 ea	5 ea	10 ea	15 ea	20 ea	25 ea
Tubes (**11.2µl aliquot** for **15µl rxn**. volume)	3 ea	15 ea	30 ea	45 ea	60 ea	75 ea

3. Aliquot each 33.6µl (for 45µl rxn volume) or 11.2µl (15µl rxn volume) master mix to 0.5ml or 1.5ml tubes on ice. After aliquot, quickly re-frozen at -70°C.

[NOTE] The activity of solution I/II is stable for at least 12 months at -70°C, however, the master mix is stable only for 60~70 days at -70°C. So, when you prepare master mix, please mix the amounts according to the table as much as you need.

4. Perform *in vitro* Transcription and Translation reaction according to the following protocol.

Master mix
: mixture of solution I/II

All step for translation should be carried out on ice.

You may scale down to 15µl reaction volume.

1. Remove the Master Mix from storage at -70°C. Rapidly thaw the prepared Master Mix by hand-warming and place on ice.

2. Assemble the reaction components in a 0.5ml or 1.5ml microcentrifuge tube on ice according to the reaction **table 2**. After adding of all the components, gently mix by pipetting. If necessary, centrifuge briefly to return the reaction to the bottom of the tube.

3. Mix well the components by pipetting, and incubate the reaction at 37°C for 60 minutes.

4. Analyze the results of translation by radiolabel incorporation, Western blot, or our F-Detector™ (under FLA scanner).

Table 2. Reaction Table for *in vitro* Transcription/Translation

45µl rxn. volume	Autoradiography		Western Blot	F-Detector™
	^{35}S-Met	^{14}C-Leu		
Master mix(1)	33.6µl	33.6µl	33.6µl	33.6µl
dH_2O(2)	11.4µl – A µl	11.4µl – A µl	11.4µl – A µl	11.4µl – A µl
Plasmid DNA(3)	A µl	A µl	A µl	A µl
^{35}S-Met(4)	1.5µl	–	–	–
^{14}C-Leu(5)	–	3µl	–	–
F-Detector™(6)	–	–	–	3µl
Total volume	**45**µl	**45**µl	**45**µl	**45**µl

(1) The Master Mix is a mixture of solution I and II.
(2) Use RNase-free water provided in Genelator™ kit.
(3) The optimal result is obtained when 1µg of plasmid DNA template is used. However, we have used 0.2~2.0µg of DNA template and obtained satisfactory levels of translation.
(4) We recommend using 10~40µCi (1~4µl) of [^{35}S]methionine can be added to the Genelator™ reactions, depending upon the balance between labeling efficiency and cost. For gene constructs that express well and contain several methionines, the 10µCi level (1.5µl) is sufficient for detection.
(5) We recommend using 15~45µCi (3~9µl) of [^{14}C]leucine can be added to the Genelator™ reactions, depending upon the balance between labeling efficiency and cost. For gene constructs that express well, the 15µCi level (3.0µl) is sufficient for detection.

Master mix
: mixture of solution I/II

All step for translation should be carried out on ice.

You may scale down to 15µl reaction volume.

You can visualize the EGFP protein expression as a positive control (supplied) under UV transilluminator or UV-lamp. Refer to the following protocol and data.

1. Remove the Master Mix from storage at -70°C. Rapidly thaw the prepared Master Mix by hand-warming and place on ice.

2. Assemble the reaction components in a 0.5ml or 1.5ml microcentrifuge tube on ice according to the reaction **table 3**.

3. Mix well the components by pipetting, and incubate the reaction at 37°C for 60 minutes.

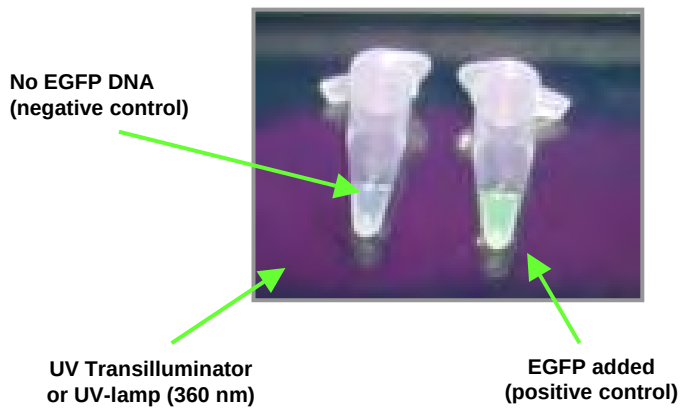
4. Analyze the results of translation on UV-transilluminator or UV-lamp.

Table 3. Positive Control Reaction

	15µl Reaction	**45µl Reaction**
Master mix	11.2µl	33.6µl
Control EGFP[(1)]	3.8 µl (max)	11.4 µl (max)
Total volume	**45**µl	**45**µl

[(1)] You can add a positive control EGFP DNA less than 3.8µl or 11.4µl as shown in Table 2.

UV visualization of enhanced green fluorescent protein (EGFP) produced using Genelator™ *in vitro* Transcription/Translation Mini Kit. In vitro protein synthesis without DNA template (***left tube***), and with EGFP positive-control DNA (***right tube***).



[cf] The GFP needs molecular oxygen to form the fluorophore post-translationally. The yield of properly-folded fluorescent GFP is further increased by storing the reaction solution after the expression of 24 hours at 4°C. After 24 hours of storing at 4°C, the maturation is almost completed.

SDS-PAGE & Fluorescence Scanning (FLA Device)

Genelator™ DESCRIPTION

Using **Genelator™** ***in vitro*** **Transcription/Translation Kit**, target protein could be produced as a soluble form in large-scale quantity compared to conventional commercial cell-free translation kit. The **Genelator™ Kit** is a simple one-pot type system including T7 RNA polymerase and is suitable for the production of aggregation-prone protein originating from eukaryote as a soluble form. And **Genelator™ Kit** serves as an alternative method for *in vivo* expression, especially for toxic proteins and/or protease sensitive proteins.

Genelator™ DATASHEET

❖ **EPO (Erythropoietin) Production by Genelator™ Kit**

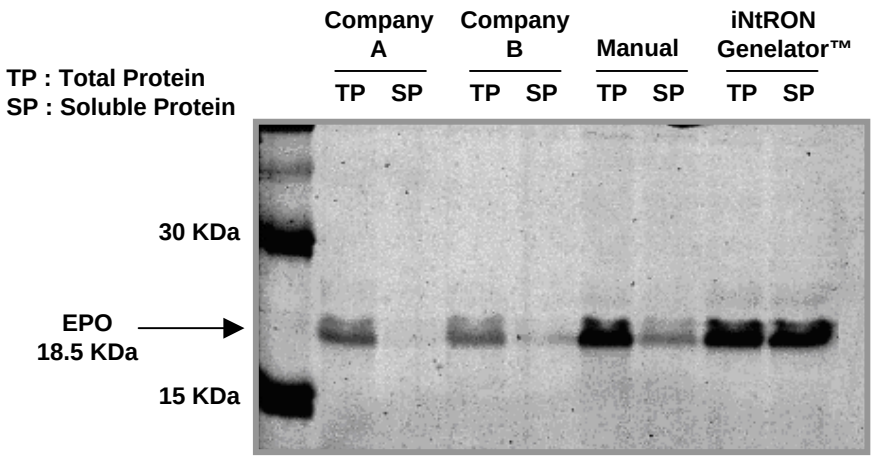
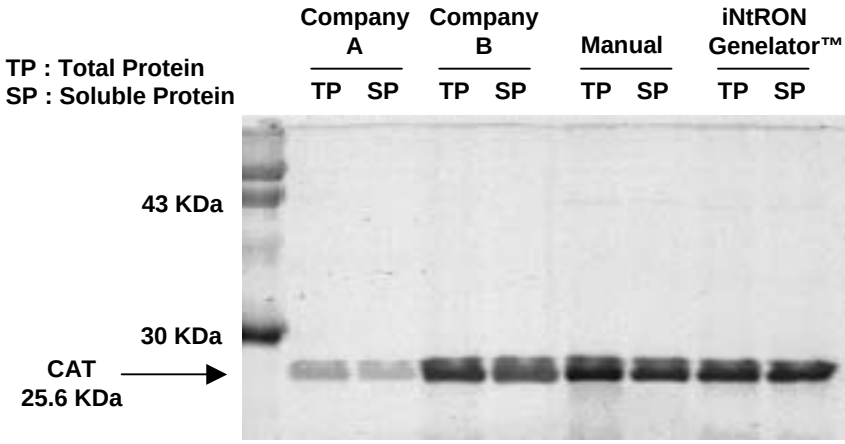


Fig 1. SDS-PAGE and Fluorescent Scanning of Erythropoietin (EPO)
The EPO protein was produced in several cell-free protein synthesis systems and labeled using F-Detector™ Kit. EPO is a eukaryotic protein which is produced in biologically inactive and inclusion body when it is expressed in bacteria.
TP : Total protein produced via various Cell-Free System
SP : Soluble protein (in the supernatant after centrifugation ; 10,000×g, 10min.) produced in various Cell-Free System
Company A : commercial cell-free protein synthesis kit
Company B : commercial cell-free protein synthesis kit
Manual : conventional cell-free protein synthesis method prepared by us
Genelator™ : Genelator™ *in vitro* Transcription/Translation Mini Kit

❖ CAT and GFP Production by Genelator™ Kit

(CAT, Chloramphenicol Acetyl Transferase; GFP, Green Fluorescent Protein)

(1) Panel A : CAT Protein



(2) Panel B : GFP Protein

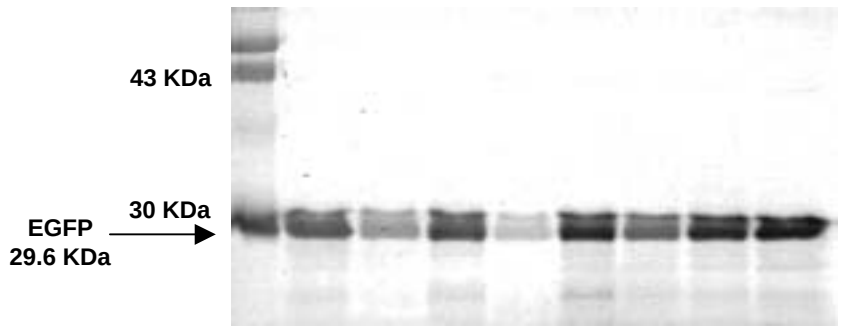


Fig 2. SDS-PAGE and Fluorescent Scanning of Chloramphenicol Acetyl Transferase (CAT, panel A) and Green Fluorescent Protein (GFP, panel B)
The CAT is a cytoplasmic soluble protein, and GFP protein is a natural fluorescence detectable protein. The translated proteins were labeled using F-Detector™ Kit.
TP : Total protein produced via various Cell-Free System
SP : Soluble protein (in the supernatant after centrifugation ; 10,000×g, 10min.) produced in various Cell-Free System
Company A : commercial cell-free protein synthesis kit
Company B : commercial cell-free protein synthesis kit
Manual : conventional cell-free protein synthesis method prepared by us
Genelator™ : Genelator™ *in vitro* Transcription/Translation Mini Kit

SDS-PAGE & Coomassie Brilliant Blue Staining

❖ **SDS-PAGE and Coomassie Brilliant Blue Staining**

(DHFR, CAT, and EGFP)

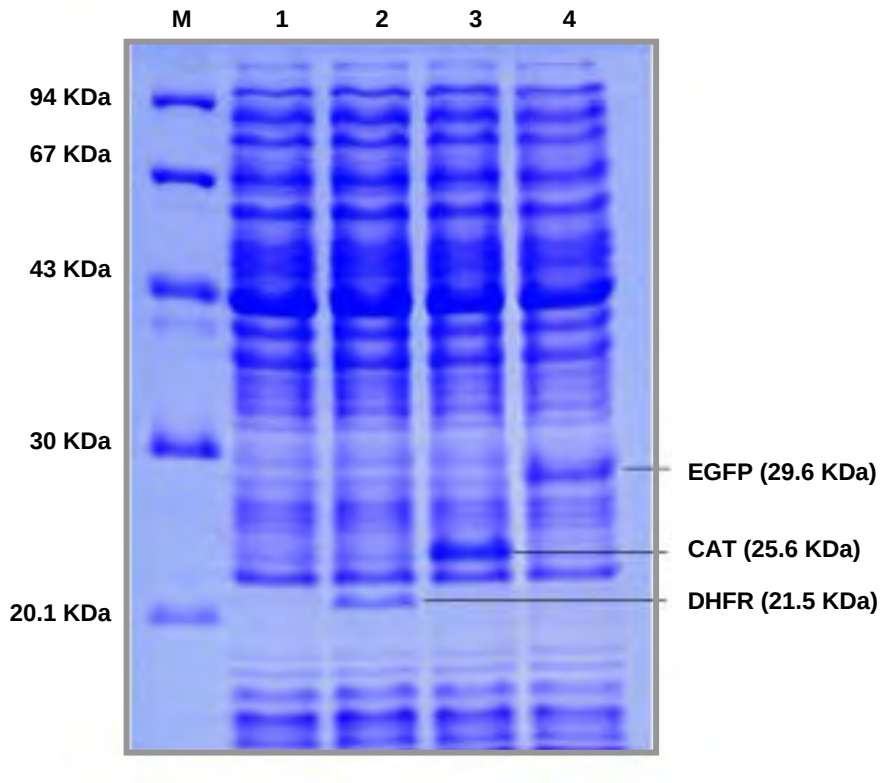


Fig 3. SDS-PAGE and Coomassie Brilliant Blue (CBB) Staining of Three Kinds of Proteins Synthesized by Genelator™ Kit

After synthesizing proteins with Genelator™ kit, the SDS-PAGE (12%) was performed, and the gel was stained with CBB according to standard protocol.

Lane 1 : Negative control (No DNA template)

Lane 2 : DHFR (21.5 KDa, Dihydrofolate reductase) plasmid DNA addition

Lane 3 : CAT (25.6 KDa) plasmid DNA addition

Lane 4 : EGFP (29.6 KDa) plasmid DNA addition

❖ **SDS-PAGE and Coomassie Brilliant Blue Staining (β-gal and GroEL)**

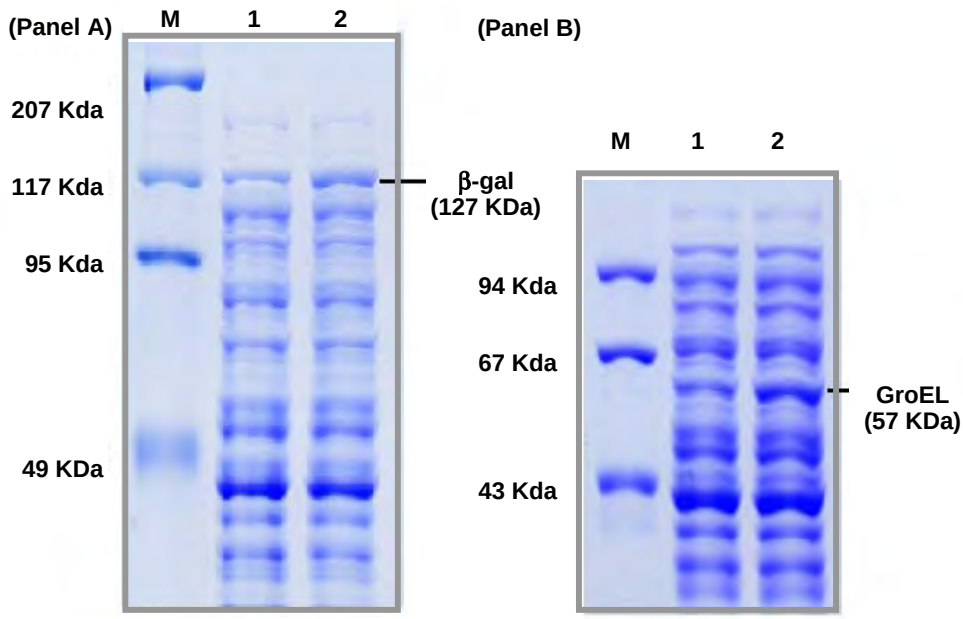


Fig 4. SDS-PAGE and Coomassie Brilliant Blue (CBB) Staining of Two Kinds of Proteins Synthesized by Genelator™ Kit

After synthesizing proteins with Genelator™ kit, the SDS-PAGE was performed, and the gel was stained with CBB according to standard protocol.

[PANEL A]

Lane 1 : Negative control (No DNA template)

Lane 2 : β-gal (127 KDa, β-galactosidase) plasmid DNA addition

[PANEL B]

Lane 1 : Negative control (No DNA template)

Lane 2 : GroEL (57 KDa, Chaperonin) plasmid DNA addition

Co-Expression of Multiple Proteins in a Single Reaction

❖ **SDS-PAGE and Coomassie Brilliant Blue Staining**

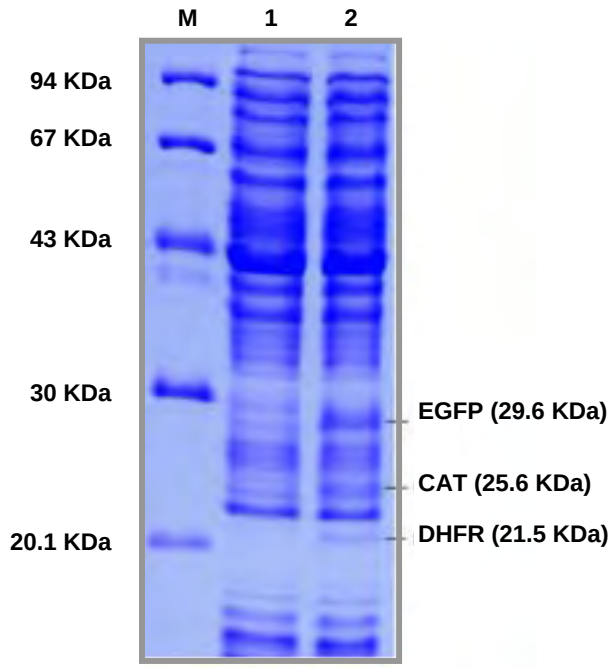


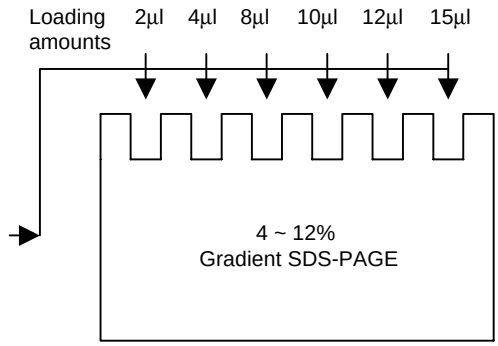
Fig 5. Co-expression of DHFR, CAT and EGFP in a Single Reaction Tube
For co-expression of multiple proteins, the cell free protein synthesis reaction was performed by using Genelator™ kit in a single reaction tube with DHFR, CAT, and EGFP plasmids. After *in vitro* synthesizing proteins, SDS-PAGE (12%) and CBB staining were performed.
Lane 1 : Negative control (No DNA template)
Lane 2 : EGFP, CAT, and DHFR DNAs addition in a single reaction tube

SDS-PAGE & Western Blot Analysis

❖ Western Blot Analysis

After performing *in vitro* translation reaction, you can easily detect the protein expression by general Western Blot Analysis.

Lane	Test
Master mix	**33.6**µl
dH_2O	**0** µl
EGFP DNA(200ng/µl)	**11.4** µl
Total volume	**45.0** µl



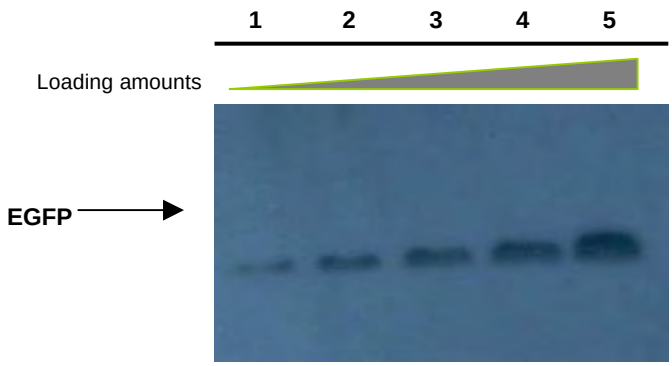


Fig 6. SDS-PAGE and Western Blot Analysis
The EGFP protein was produced in several cell-free protein synthesis systems and performed Western Blot Analysis with anti-EGFP Ab.

1° Ab → 2000:1
(anti-His-Tag Ab)
2° Ab → 2000:1
(HRP-tagged Ab)

Western Detection
: WEST-ZOL™ plus
→ Cat. 16021

The Genelator™ Troubleshooting guide may be helpful in solving some problems that may occur. If you have other problems without this handbook, please contact our Technical Service Department.

Comments & Suggestions

❖ **No control protein visible**

a) Nuclease contamination	Use RNase- and DNase-free tubes and tips.
b) Storage condition	The kits must be stored at -70°C or -20°C.
c) Loss of activity	Check the expiration date, and storage condition

❖ **No target protein visible**

a) Poor quality of DNA	Please use iNtRON DNA-spin™ plasmid DNA purification kit. Avoid adding excessive salts or glycerol when adding template DNA. The activity may be inhibited by NaCl (>50mM), glycerol (>1%), or by very small amounts of Mg^{2+}, or potassium salts.
b) Wrong quantity of DNA	Check the concentration and integrity of the DNA template. Titrate the amount of DNA used in the *in vitro* translation to determine the optimal amount.
c) RNase contamination	Omit RNase addition step in plasmid preparation
d) Error in promoter	Check the promoter of vector DNA. Make sure that the promoter is T7-based or Lac-based promoter.

❖ **Low expression yield**

a) Nuclease contamination	Use RNase- and DNase-free tubes and tips.
b) Poor quality of DNA	Please use iNtRON DNA-spin™ plasmid DNA purification kit. Do not add RNase into Lysis solution. Use both Washing Buffer A and B.
c) Wrong quantity of DNA	Check the concentration and integrity of the DNA template. Add fully plasmid DNA without dH_2O into reaction tube.
d) Lac promoters	Add 1mM IPTG to translation reaction to overcome endogenous lac repressor.
e) RNase contamination	Omit RNase addition step in plasmid preparation

❖ **Low yield of active protein**

a) Deficiency of post-translational modification	*E. coli* lysate can not introduce posttranslational modifications like glycosylation, phosphorylation, or signal-peptide cleavage.
b) Necessity of disulfide bond formation	Genelator™ contains reducing agents (equivalent to about 2mM DTT). For promotion of disulfide bond formation, dialysis of cell-free reaction sample to appropriate refolding buffer, or dilution of cell-free reaction sample with appropriate refolding buffer.

MATERIAL PROVIDED

For Laboratory Use. The F-Detector™ kit contains sufficient reagents to perform approximately 20 times × 45µl translation reactions. Includes :

Material Provided	Quantity
(1) F-Detector™ Solution[1] (Fluorescent tRNA)	60 µl × 1 ea.
(2) Handbook Guide	Protocol

[1] <3.0µl per 45µl Genelator™ reaction volume

INTELLECTUAL PROPERTIES

This **F-Detector™** has been co-developed with DreamBiogen, Co. (www.dreambiogen.com). Furthermore, Materials in this application and Methods of use are covered by various intellectual Properties.

Trademarks

Genelator™, F-Detector™, DNA-spin™, DNA-midi™, MEGA-spin™, PCRquick-spin™

Patent & Patent Pending

KR10-24695	KR10-131166	KR10-401296	KR10-399337	US5,593,856
KR10-009628	PCT/KR2004/000302		JP2001-47494	JP2001-57514
US09/783,721	US09/792,647	EP01420032.3	EP01420050.0	

STORAGE and STABILITY

The **F-Detector™ Cell Free Labeling Kit** is shipped on dry ice. All components must be **stored at below –70 ℃**. When stored under the above conditions and handled correctly, the kit can be kept for 12 months without showing any reduction in kit performance.

PRODUCT USE LIMITATION

The Genelator™ kit is developed, designed, and sold for research purposes only. It is not to be used for human diagnostic or drug purposes or to be administered to humans. All due care and attention should be exercised in the handling of many of the materials described in this text.

You must have FLA device for F-Detector™.

Many protein modification systems either post-translational or post-synthetic modification have been developed. But, these methods have not been able to label the growing nascent proteins selectively during their synthesis for detection and are unsuitable for selective labeling of the produced protein in a pre-existing protein mixture. A variety of techniques have been investigated for labeling the nascent protein during their synthesis. There are two major classes of commonly used detection methods. The translated proteins are detected based on radioactivity or biotin moiety. The two complementary detection systems are useful for screening of expressed proteins. One of the most common methods used in the detection of proteins synthesized or characterized using in vitro translation systems is the incorporation of radioactively labeled amino acids such as [35S]Methionine or [14C]Leucine. Safety, regulatory issues, waste disposal, and lengthy exposure times are the primary drawbacks associated with the use of radioactivity. The use of radioactivity is undesirable for routine laboratory applications as well as for the automated analysis of samples. As with radioactive incorporation, in the method based on biotin moiety, reactions containing proteins are resolved on SDS-PAGE gels and the proteins must be electroblotted to a solid matrix such as nitrocellulose or PVDF membranes. And electroblotted proteins can be detected non-isotopically using either colorimetric or chemiluminescent detection reagents.

F-Detector™ Cell Free Labeling Kit serves a sensitive, non-isotopic, fluorescence-based method for the detection of nascent proteins directly in gels with high signal-to noise ratios after in vitro synthesis. A fluorescent technique using F-Detector™ Cell Free Labeling Kit offers a simplified alternative method that dramatically reduces the time required to obtain data due to the rapid detection of the protein bands immediately after electrophoresis without any downstream processing and eliminates manipulations associated with radioactivity (i.e., gel fixing and drying or transferring to a membrane). It also eliminates the requirement for electroblotting associated with other non-isotopic technologies based on the biotin moiety.

Under the optimal conditions, fluorescent bands from nanogram levels of in vitro-produced proteins could be detected directly from gels using a conventional UV-transilluminator. Higher sensitivity (~100-fold) can be obtained using a laser-based fluorescent gel scanner. Fluorescent marker in F-Detector™ Cell Free Labeling Kit can be excitable over a wide range of wavelengths for detection with both an UV-transilluminator (gel documentation system) and a laser-based fluorescent gel scanner.

The ability to rapidly synthesize nascent proteins containing a fluorescent reporter group facilitates many biotechnological applications including functional analysis of gene products, drug discovery, and mutation screening. For example, nascent proteins labeled with fluorophore should be suitable for detection by laser-induced fluorescence (LIF) in conjunction with capillary electrophoresis (CE). This technology can be also be used to rapidly screen for protein truncation products which are produced from defective mutant genes or for the interaction

between a target in vitro produced protein and other molecules such as in the screening for drug candidates. Other possible application of F-Detector™ Cell Free Labeling Kit include in vitro expression cloning (IVEC), in vitro selection of proteins, and functional gene screening based on an antisense display. F-Detector™ Cell Free Labeling Kit could be readily used for high-throughput screening to identify protein expression and examine its function.

❖ **F-Detector™ Cell Free Labeling Kit**

CAT. NO. 12021
Total 60µl (< 1 ~ 3µl per 45µl Genelator™ rxn.)
(1) F-Detector™ fluorescent tRNA one tube containing 60µl mixture (2) Handbook Guide

DESCRIPTION

F-Detector™ Cell Free Labeling Kit serves a sensitive, non-isotopic, fluorescence-based method for the detection of nascent proteins directly in gels with high signal-to noise ratios after *in vitro* synthesis. A fluorescent technique using **F-Detector™ Kit** offers a simplified alternative method that dramatically reduces the time required to obtain data due to the rapid detection of the protein bands immediately after electrophoresis without any downstream processing and eliminates manipulations associated with radioactivity (*i.e*., gel fixing and drying or transferring to a membrane). It also eliminates the requirement for electroblotting associated with other non-isotopic technologies based on the biotin moiety.

STORAGE RECOMMENDATION

Always avoid multiple freeze-thaw cycles (do not subject to more than 5 freeze-thaw cycles) or exposure to frequent temperature changes. These fluctuations can greatly alter product stability. Dispense the product into smaller aliquots as needed.

STORAGE & STABILITY

It is recommended the product to be stored at –70 ℃ or –20 ℃ at which it is stable for at least 6 months.

PRECAUTIONS

The F-Detector™ and Genelator™ Kit is for laboratory research use only.

KIT CONTENTS

This product contains a fluorescent modified tRNA. This fluorescent modified tRNA allows the fluorescent detection of proteins synthesized in cell-free protein synthesis systems. Fluorescent dye used for this labeling is 5(6)-carboxyfluorescein [5(6)-FAM; Excitation maximum: 493nm; Emission maximum: 520nm; Molar extinction coefficient: 78,600 $cm^{-1}M^{-1}$] (Figure 11).

MATERIALS

- Genelator™ *in vitro* Transcription/Translation Kit
- F-Detector™ Cell Free Labeling Kit
- Plasmid DNA template (high purity) encoding the protein of interest
- Water-bath, or heating block

■ The F-Detector™ Kit is extremely sensitive to nuclease contamination. Always use RNase- and DNase-free reaction tubes and pipet tips.

■ The fluorescent modified tRNA in F-Detector™ Kit is sensitive to multiple freeze-thaw cycles. The kit is provided as 60µl individual aliquots in a single tube. Once thawed, use F-Detector™ Solution within 2 hours. Do not refreeze and thaw more than five times. Dispense the product into smaller aliquots as needed.

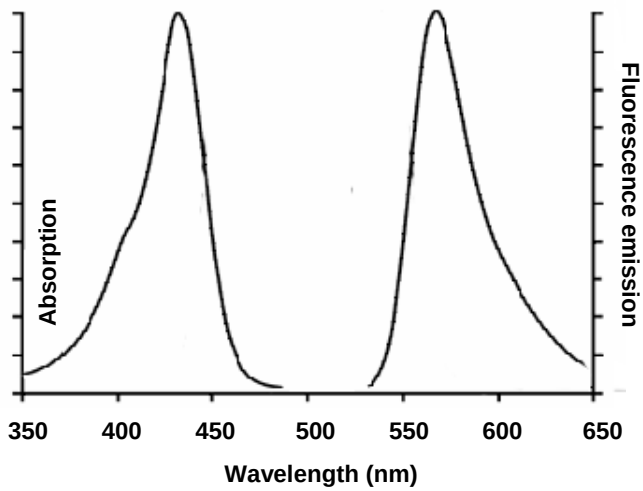
■ This system is also extremely sensitive to RNase. Please no RNase addition step to general Sol I solution during the purification step of plasmid DNA.

■ The plasmid DNA suitable for this system is T7 promoter based or Lac promoter based vector system. If you use other promoter based vector systems such as SP6 promoter, please add an suitable RNA polymerase.

■ Except for the actual transcription-translation incubation (37 ℃), all handling steps should be carried out on ice (important).

■ The recommended incubation temperature for protein synthesis is 37 ℃, but lower incubation temperatures may improve protein solubility in some cases.

Fig 7. Absorption & Fluorescence Emission Spectra of 5(6)-Carboxyfluorescein



As described, **F-Detector™ Cell Free Labeling Kit** serves a sensitive, non-isotopic, fluorescence-based method for the detection of nascent proteins directly in gels *in vitro* protein synthesis.
You can use this **F-Detector™ Kit** in various *in vitro* transcription/translation systems from other companies. This labeling kit enables to easily detect your targeted protein without using an radioisotope. During *in vitro* transcription/ translation reaction, you may co-incubate after adding an appropriate amount of **F-Detector™** solution. After finishing SDS-PAGE, you can easily detect your target protein synthesis on laser-base fluorescence scanning (FLA) device.

Adding Amounts of F-Detector™

We recommend to add only 0.5~1µl of F-Detector™ solution per 15µl *in vitro* transcription/ translation reaction. You may add proportionally more F-Detector™ solution according to your reaction volume. The F-Detector™ solution does not affect to protein synthesis yield itself during *in vitro* transcription/translation reaction.
More and more adding F-Detector™ solution to your reaction, the signal of expression may be only strong.

The following data shows the signal density according to the amounts of F-Detector™ solution.

Table 4. Pipetting Scheme for F-Detector™ Kit

Components	15µl Reaction Volume			45µl Reaction Volume		
	User Sample	Positive control	Negative control	User Sample	Positive control	Negative control
Master Mix	11.2µl	11.2µl	11.2µl	33.6µl	33.6µl	33.6µl
DNA Template	0.1µg ~ 0.5µg	<2.0µl	×	0.5µg ~ 2.0µg	<5.0µl	×
F-Detector™ Kit	0.5~1.0µl	0.5~1.0µl	0.5~1.0µl	1.0~3.0µl	1.0~3.0µl	1.0~3.0µl
RNase-Free Water	Add to 15µl volume	Add to 15µl volume	Add to 15µl volume	Add to 45µl volume	Add to 45µl volume	Add to 45µl volume

The Genelator™ Troubleshooting guide may be helpful in solving some problems that may occur. If you have other problems without this handbook, please contact our Technical Service Department.

Comments & Suggestions

Protein insolubility	
Protein aggregation	We recommended a 37°C incubation for protein synthesis, but lower incubation temperatures may improve protein solubility.
Degradation of protein	
a) Premature termination	Check the sequence of the target gene (open reading frame, mutation yielding a stop codon)
b) Protease contamination	Add EDTA-free protease inhibitors to the reaction.
No Fluorescence Signal	
a) Error in protein synthesis	Check the expression of positive control DNA (EGFP).
b) Low F-Detector™ amount	Add more F-Detector™ in case of low expressed protein
c) Storage condition	The kits must be stored at -70°C or -20°C.
d) Poor quality of DNA	Please use iNtRON DNA-spin™ plasmid DNA purification kit.
e) Wrong quantity of DNA	Check the concentration and integrity of the DNA template. Titrate the amount of DNA used in the *in vitro* translation to determine the optimal amount.
f) Lac promoter	Add 1mM IPTG to translation reaction to overcome endogenous lac repressor.
g) Premature termination	Check reading frame of DNA template (eg., stop codon mutation)
h) Protease contamination	Add EDTA-free protease inhibitors to the reaction.
i) Kit expired	Check the expiration date.
j) Weak fluorescent intensity	Leaving the fluorescent-labeled in vitro translation products alone for a long period (> 10 hrs) may decrease the signal intensity due to its hydrolysis. The labeled proteins prepared by F-Detector™ kit should be also handled according to the protocol.