TRANSGEN BIOTECH

TRANSGEN

K10 Excellence

10 Outstanding Kits for Your Research Needs

Providing Innovative Reagents for Life Sciences since 2006

TransZol Up Plus RNA (ER501)

Description

TransZol Up Plus RNA Kit is suitable for the isolation of total RNA from **cells and tissues**. *TransZol* Up Plus RNA Kit not only has features of **strong lysis capability, high yield and a wide range of applications** of *TransZol* Up, but also the feature of **high purity** of spin column extraction.

Features

- Strong lysis capability.
- Wide range of applications: animal and plant tissues, cells, blood, bacteria, viruses, etc. Small samples (50-100 mg tissue, 5 x 10^6 cells, 200 µl blood). Large samples (≥1 g tissue or ≥10^7 cells).
- High yield: the binding capacity of the spin-columns is up to 100 µg RNA.
- High purity: minimum DNA and protein contamination.

1. Purpose of experiment

Test the RNA extraction effect of ER501 on various of samples.

2. Experimental materials

2.1 Samples

Tobacco leaves, mouse liver, Hela cells

2.2 Reagents

TransZol Up Plus RNA (ER501)

TransScript® Green One-Step qRT-PCR SuperMix, TransGen, AQ211

*Trans*2K® Plus II DNA Marker, TransGen, BM121

2.3 Instruments

NanoDrop Spectrophotometer, Fluorescent Quantitative PCR Instrument

3. Experiment result

3.1 Result of total RNA extraction

3.1 .1 RNA extraction of tobacco leaves

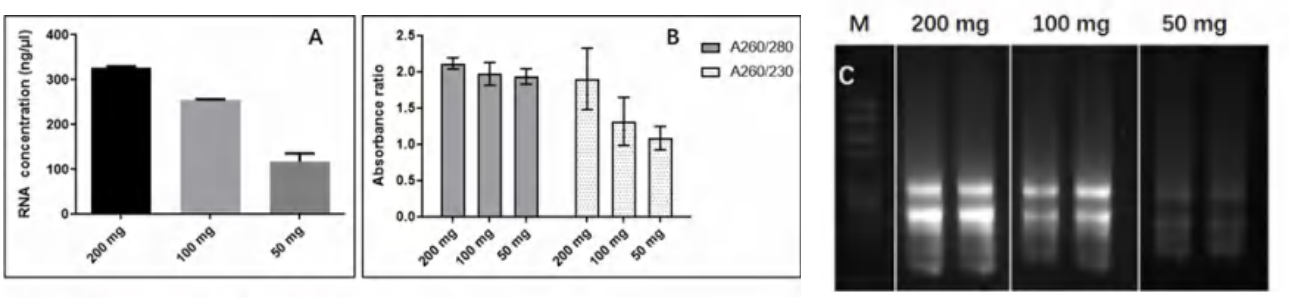


Fig. 1. Extract RNA from 50-200 mg of tobacco leaves using ER501 kit

A. Extracted total RNA concentration (elution volume is 50 µl); B. Absorbance ratio of A260/280 and A260/230 of the extracted product; C. Agarose gel electrophoresis of the extracted product (loading volume of each well is 5 µl). M: DNA Marker

The extraction results showed that ER501 had a good extraction yield for 50 - 200 mg tobacco leaves. The A260/280 ratio of extracted RNA at different sample amounts was stable, all around 2.0, while the A260/230 ratio gradually decreased as the extraction yield decreased, which was in line with expectations. The electrophoresis gel images showed that the integrity of the extracted product bands at each sample amount was good, and there was no obvious degradation.

3.1.2 RNA extraction of mouse liver

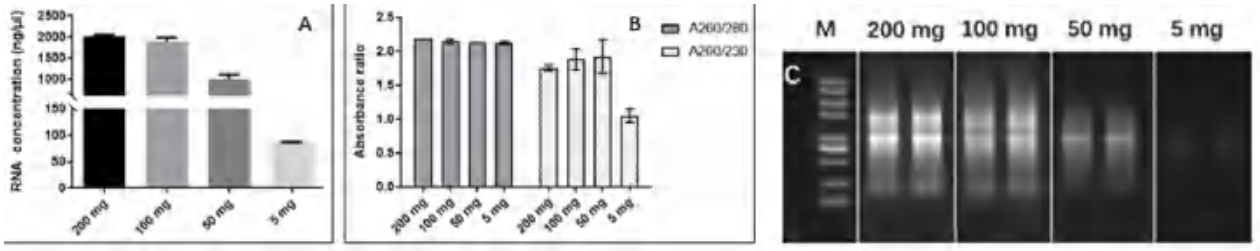


Fig. 2. Extract RNA from 5-200 mg of mouse liver using ER501 kit

A. Extracted total RNA concentration (elution volume is 50 μl); B. Absorbance ratio of A260/280 and A260/230 of the extracted product; C. Agarose gel electrophoresis of the extracted product (loading volume of each well is 5 μl). M: DNA Marker

The results showed that the maximum input for mouse liver using ER501 was about 100 mg, and increasing the sample amount on this basis had no effect on RNA yield. The A260/280 ratio of the extracted RNA was stable between 2.1-2.2; the average A260/230 of extraction product from 50 mg sample was close to 2.0. The electrophoresis gel image showed that the band integrity of the extracted product was good at each sample amount, and there was no obvious degradation.

3.1.3 RNA extraction of Hela cells

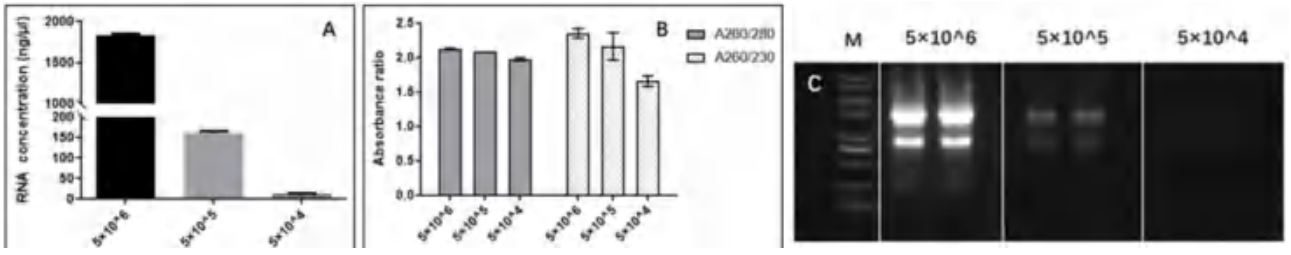


Fig. 3. Extract RNA from 5×10^4~5×10^6 Hela cells using ER501 kit

A. Extracted total RNA concentration (elution volume is 50 μl); B. Absorbance ratio of A260/280 and A260/230 of the extracted product; C. Agarose gel electrophoresis of the extracted product (loading volume of each well is 5 μl). M: DNA Marker

The results showed that the RNA yield was in direct proportion to the cell amount, and the A260/280 ratio was all greater than 2.0, and the A260/230 ratio was all greater than 1.6. The electrophoresis gel image showed that the integrity of the extracted product bands was good at each sample amount, and there was no obvious degradation.

3.2 Downstream qPCR detection

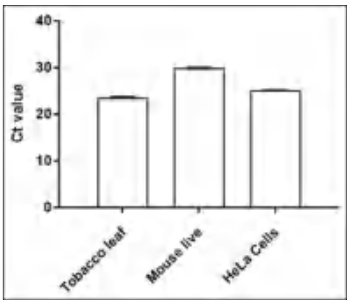


Fig. 4. The results of the quality test of the extracted products using the AQ211 kit with cDNA-specific primers of tobacco, mouse, and human internal reference genes

In the experiment, the RNA of tobacco leaves was extracted from 100 mg sample, the RNA of mouse livers was extracted from 50 mg sample, the RNA of Hela cells was extracted from 5×10^5 samples.
The input amount of qPCR template was all 50 ng.

The quality of extracted RNA directly affects the results of downstream experiments. This test uses the qPCR method to indirectly test the inhibitor levels of extracted RNA from tobacco leaves, mouse liver, Hela cells and other samples. The results showed that when 50 ng RNA was input, the average Ct value of tobacco leaves was 23.64, the average Ct value of mouse liver was 29.88, and the average Ct value of Hela cells was 25.10.

4. Summary

Based on the above content, the test is summarized as follows:

4.1 Wide range of sample extraction.

ER501 is suitable for extracting a wide range of samples. Good extraction results were obtained for tobacco leaves, mouse liver and Hela cell samples.

4.2 The extracted product has high purity.

The inhibitor level of the extracted product was indirectly detected by qPCR method, and the amplification result was good, without significant inhibition.

4.3 The bands in some extractions showed that the 28S:18S ratio was lower than 2.0, which may be related to the sample state and sampling site.

EasyPure® Universal Plant Total RNA Kit (ER302)

Description

The kit is designed for the isolation of RNA from various fresh and dried plant samples, including plant samples rich in polysaccharides and polyphenols.

Features

- Wide range of applications: applicable to various plant tissues, especially those rich in polysaccharides, polyphenols or starch
- Fast operation: high-quality genomic RNA can be extracted in less than 30 minutes
- Safe and low toxicity: no toxic organic reagents such as phenol and chloroform
- High purity: the unique technology can efficiently remove impurities such as pigments, polyphenols and polysaccharides in the sample

1. Materials

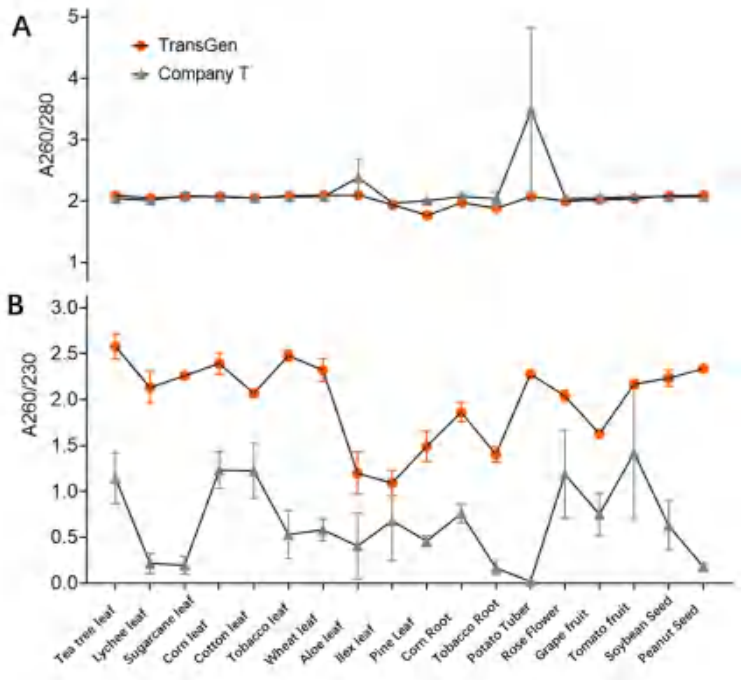
1.1 Sample

Tobacco leaves, mouse liver, Hela cells

Type of Sample	Species
Leaf	Tobacco, Wheat, Corn, Tea tree, Lychee, Sugarcane, Cotton, Ilex, Aloe, Pine
Root	Corn roots, Tobacco roots
Tuber	Potato
Flower	Rose
Fruit	Grape, Tomato
Seed	Soybean, Peanut

2. Results

2.1 Extraction performance on different type of samples



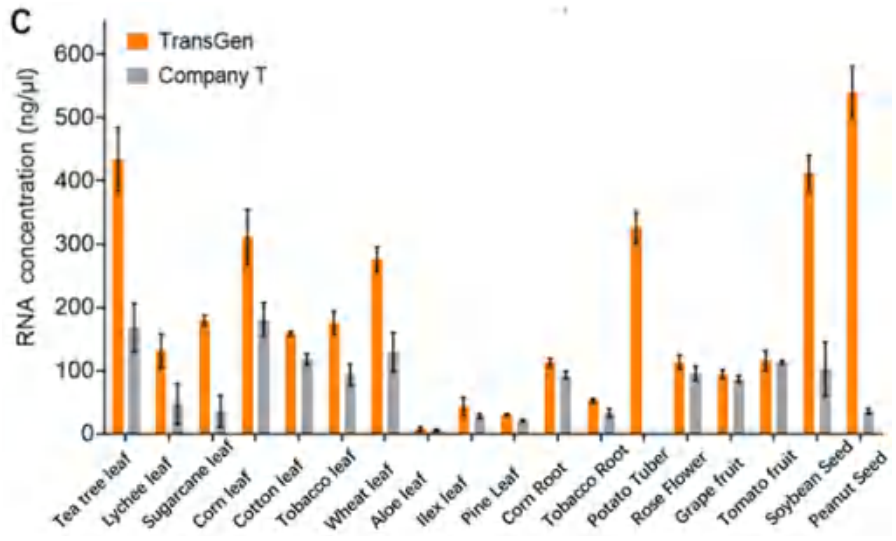


Figure 1. The extraction outcomes of ER302 (TransGen) and competitor Company T for various plant sample types were compared.

A. The A260/280 absorbance ratio of the extracted RNA; B. The A260/230 absorbance ratio of the extracted RNA; C. The concentration of the extracted RNA (Qubit)

Comparative analysis of extraction results against competitor reveals that our ER302 product demonstrates significantly superior overall performance in extraction, notably for leaf, root, and seed samples. Specifically, the competitor was unable to detect RNA concentrations from potato root samples, whereas our product consistently extracts high-quality RNA exceeding 300 ng/μL. Regarding extraction quality, the competitor's measurements for samples with higher metabolite levels exhibited substantial fluctuations, all showing A260/230 ratios around 1.0. In contrast, our ER302 product consistently produces higher-quality extraction outputs, with A260/280 ratios predominantly around 2.0 and overall superior A260/230 values compared to those of the competitor.

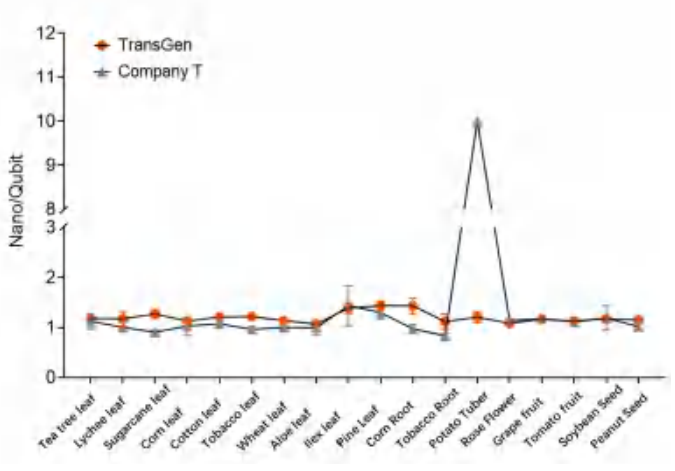


Figure 2. The extraction outcomes of ER302 (TransGen) and competitor Company T by NanoDrop and Qubit were compared.

As Company T's product failed to extract RNA from the potato tuber samples, the Nano/Qubit value neared infinity. For clarity, the results of this sample were set as 10 for illustration purposes.

The NanoDrop nucleic acid detector operates on ultraviolet absorption, with the accuracy of its RNA content detection being notably impacted by impurities in the tested sample. In contrast, the Qubit employs a specific fluorescent dye that selectively binds to RNA, yielding a more precise representation of RNA content in the tested product. Thus, the Nano/Qubit ratio can serve as an indicator of the extraction product purity. The findings reveal that, apart from the elevated reading in the potato sample extracted by Company T, the Nano/Qubit values for RNA extraction from both products exhibit fluctuations within the range of 1.0-1.2.

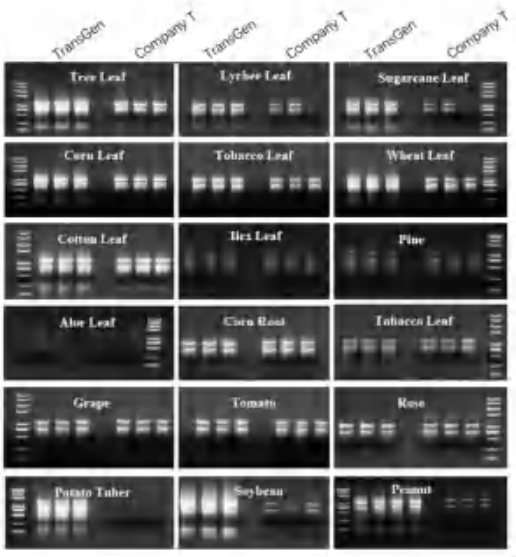
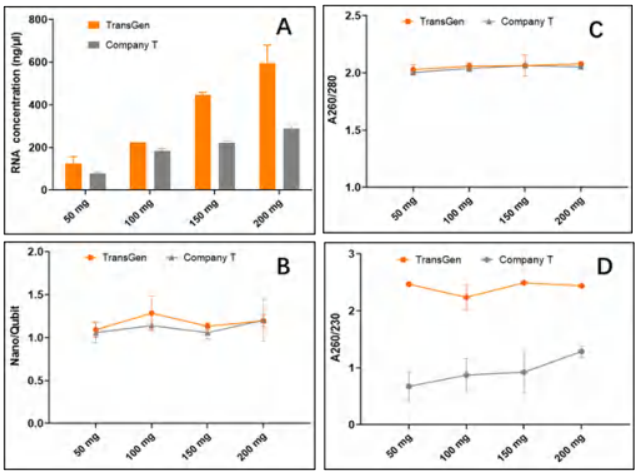


Figure 3. RNA Gel Electrophoresis of ER302 (TransGen) and competitor Company T

Electrophoresis analysis was applied to extracted RNA, the result indicates that the intensity of the bands extracted by ER302 was markedly stronger compared to Company T's product, aligning with the quantification findings (Figure 1). Additionally, the primary bands appeared well-defined and unaltered, exhibiting no apparent signs of degradation or genomic remnants. Notably, even samples with lower extraction outputs like holly leaves, pine needles, and aloe vera leaves displayed distinct primary bands.

2.2 Extraction performance with different sample quantities

2.2.1 Tea leaf (Camellia sinensis)



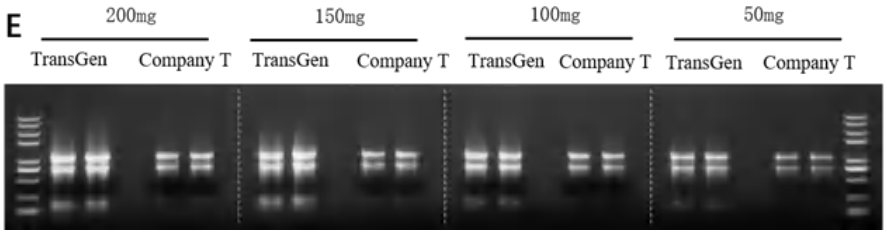
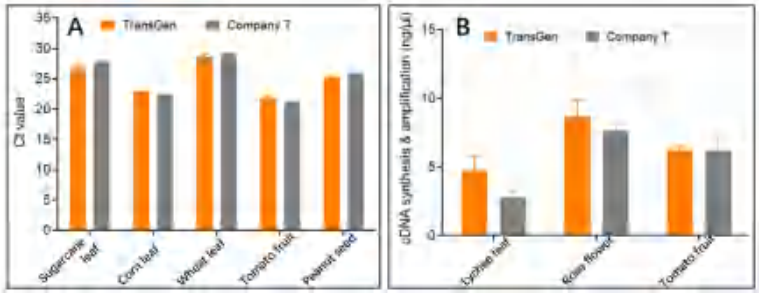


Figure 4. The extracted outcomes of tea leaf samples with varying quantities using ER302 (TransGen) and Company T products.

A. Extracted RNA concentration (Qubit); B. Ratio of NanoDrop to Qubit measurement data; C. A260/280 absorbance ratio; D. A260/230 absorbance ratio; E. Electrophoresis gel image of the extracted RNA.

Tea leaves were chosen as the test material to assess the extraction performance of the products at different sample quantities. The results indicate that ER302 demonstrates superior compatibility across varying sample mass, consistently achieving excellent extraction efficiency, even with a 200mg input, while Company T's product peaks at 150mg. Both products exhibit no notable differences in the Nano/Qubit ratio and A260/280; however, ER302 gives better A260/230 ratio surpassing 2.0. The RNA bands extracted by ER302 retain high integrity, showing no signs of degradation or genomic residues.

2.2.2 Soybean Seed



Sample Type	Product	Transcriptome library construction conditions					Yield
		Amount of RNA	mRNA Capture	Library Preparation	Method	Amplification Cycle	
Tea Leaf	Company T	500ng	EC511 (TransGen)	KP701 (TransGen)	Stranded Library	12 cycles	696
	TransGen						**834**
Wheat Leaf	Company T	500ng				12 cycles	30.9
	TransGen						**345**
Cotton Leaf	Company T	500ng				12 cycles	624
	TransGen						**684**
Rose Flower	Company T	500ng				12 cycles	864
	TransGen						**954**
Tomato Fruit	Company T	500ng				12 cycles	456
	TransGen						**924**

Figure 6. The subsequent analysis of RNA extraction by ER302 (TransGen) and Company T products.

A. qRT-PCR detection results (template amount 100ng, reaction system 20μl); B. Full-length cDNA synthesis and amplification; C. Transcriptome library construction conditions and yield.

The success of downstream experiments relies on the quality of RNA extraction. Our research incorporated three types of experiments, including qRT-PCR, full-length cDNA synthesis and

amplification, and transcriptome library construction, to evaluate the compatibility of the RNA extracted by two different products with downstream reagent kits.

By utilizing our one-step qRT-PCR kit (AQ311) to test the efficiency of reverse transcription and qPCR on the extraction products, the results indicated that both products performed comparably with no significant differences. Subsequent evaluation with our KC901 product to test the full-length cDNA synthesis and amplification capacity of the extraction products revealed a higher cDNA amplification yield with ER302, suggesting superior RNA integrity compared to Company T's product. Thus, employing ER302-extracted products for targeted gene quantification or gene cDNA fragment cloning would enhance precision and success rates. The RNA extraction, combined with our EC511 and KP701 kits for mRNA capture and library construction, exhibited notably increased library output with ER302-extracted RNA, demonstrating higher mRNA content and purity in ER302-extracted products, which ensures accurate detection of gene expression.

3. Conclusion

(1) ER302 shows exceptional extraction performance, catering to a wide range of plant sample types. Notably, its efficiency in extracting leaf, tuber, and seed tissues surpasses that of competitor significantly. ER302 can extract seed samples up to 14 times more efficiently than competitor. For potato tubers, ER302 yields high-quality RNA exceeding 300 ng/μl, while the competitor fails to produce any measurable yield.

(2) RNA extracted by ER302 demonstrates high integrity and purity, making it particularly suitable for downstream experiments such as full-length cDNA synthesis and transcriptome library construction.

(3) The extraction products exhibit no remnants of genomic DNA (gDNA). The product incorporates a gDNA removal component, effectively eradicating gDNA contamination and minimizing its influence on subsequent analyses.

EasyPure® Total RNA Kit (EC521)

Description

MagicPure® Total RNA Kit is designed for the isolation of total RNA from samples such as cultured cells, microorganisms, and animal and plant tissues using magnetic beads. This kit is **suitable for high-throughput automated nucleic acid extractor adopting magnetic rod technology.**

Product form:

Manual extraction: *MagicPure*® Total RNA Kit (EC521)
Automatic extraction: *MagicPure*® 32/96 Total RNA Kit (EC521-32/96)

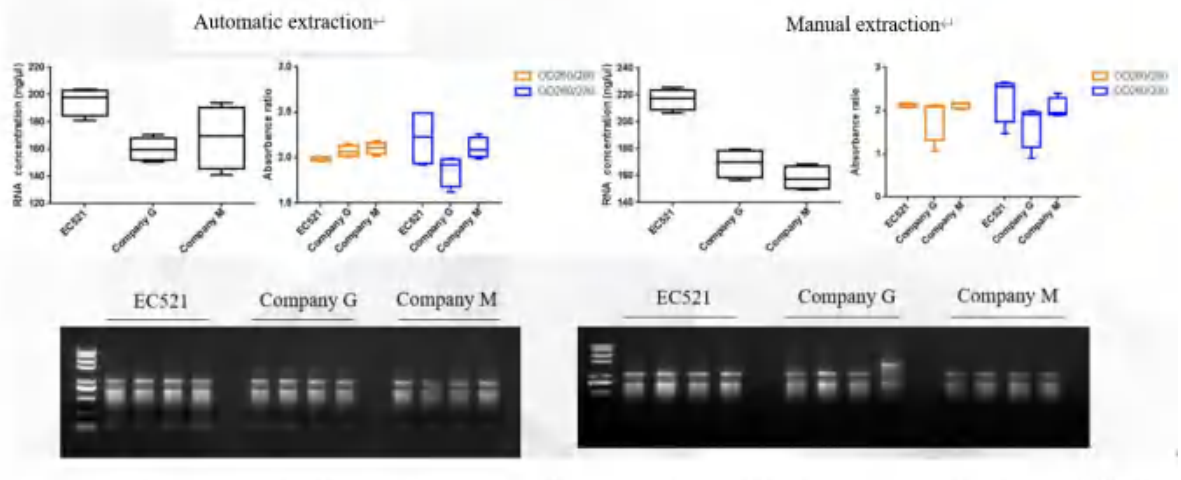
Features

- A wide range of applications: suitable for samples such as cultured cells, animal and plant tissues, viruses and bacteria.
- Easy operation: less manual operation.
- High purity: minimum DNA and protein contamination.
- Applicable to 32/96-channel high-throughput automated nucleic acid extractor adopting magnetic rod technology.

1.Wide range of applicable species

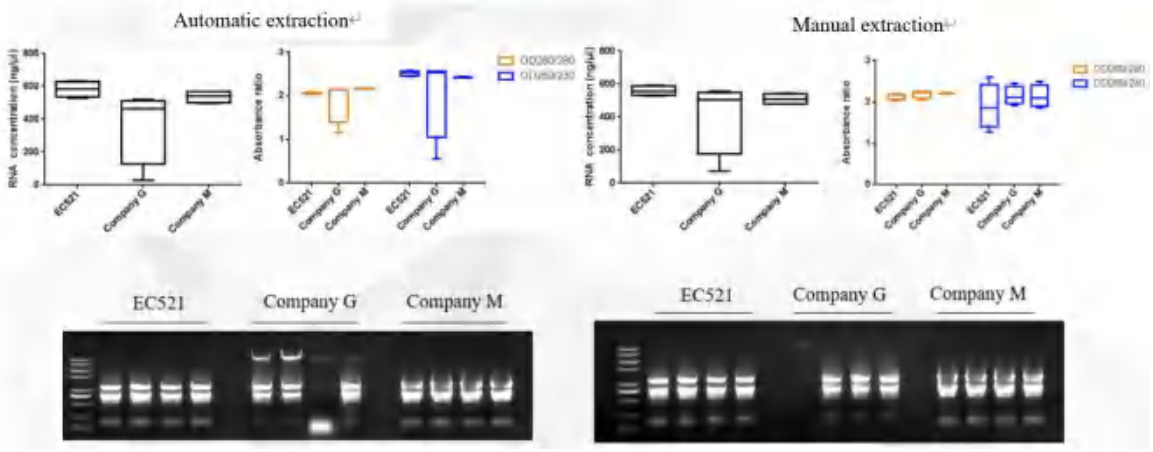
RNA was extracted from different species using reagents from TransGen, Company G and Company M.

1.1 Tobacco (100mg)



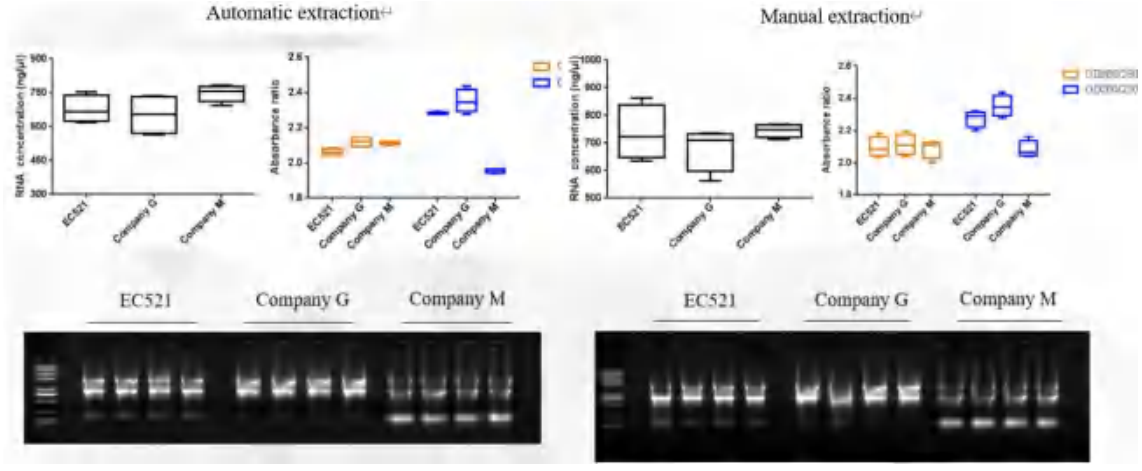
The extraction results of tobacco showed that the extraction yield of EC521 was significantly higher than that of similar products of Company G and Company M, whether it was automatic or manual version, and the extraction quality was relatively stable.

1.2 Wheat (100 mg)



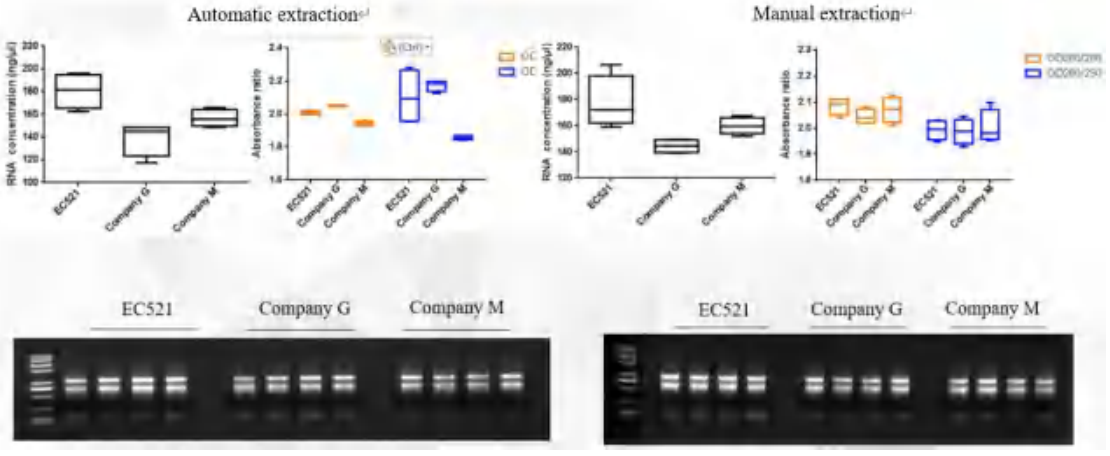
The extraction results of wheat showed that EC521 was more stable by using manual and automatic versions, and higher than the other two competing products.

1.3 Mouse liver (100mg)



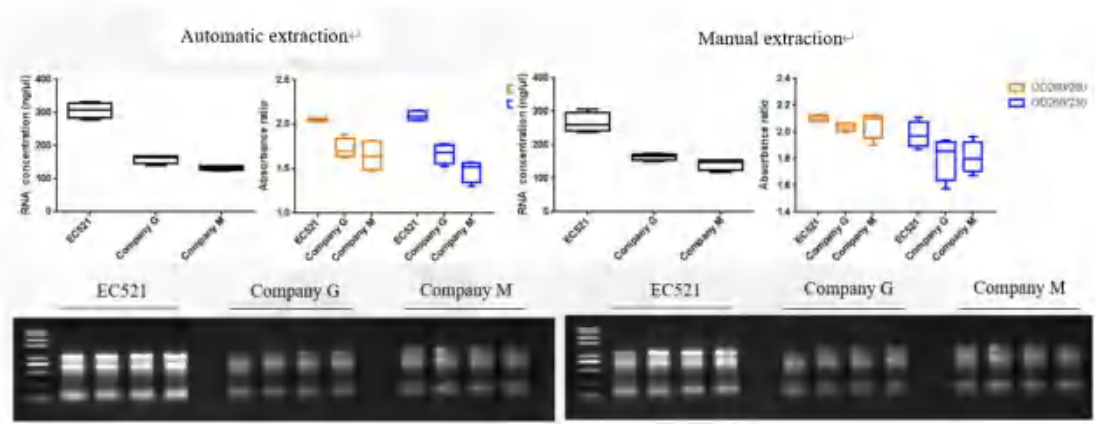
The extraction result of mouse liver showed that the extraction yield of EC521 was basically the same as that of the other two competing products, but the electrophoresis gel image showed that the three bands of the EC521 extraction product were clearer and the main band was brighter.

1.4 Hela Cells (2×10^6)



The extraction results of Hela cells showed that the manual and automatic versions of EC521 were relatively stable and higher than the other two competing products.

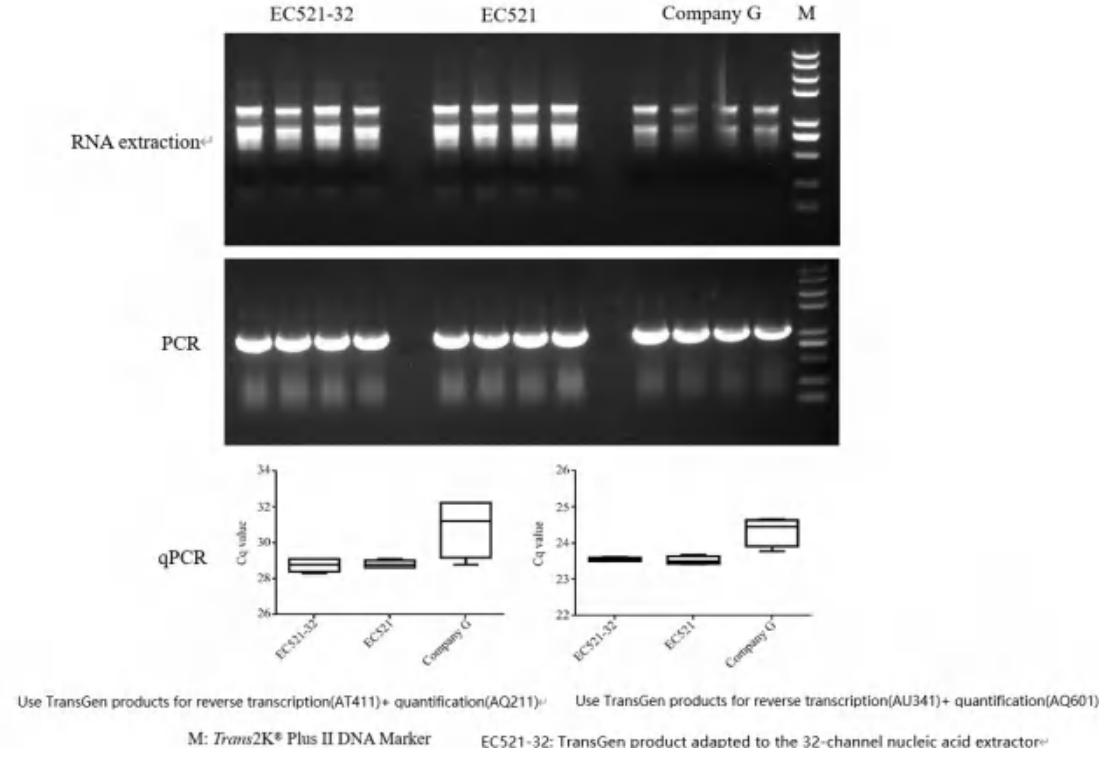
1.5 *E.Coli* (1×10^9)



The extraction results of E. coli showed that the manual and automatic versions of EC521 were significantly higher than those of the other two competitors, and the electrophoresis bands were better than those of the competitors.

2. The extracted RNA has good integrity and has no inhibition on downstream amplification

RNA was extracted from tobacco samples using TransGen and Company M products respectively, and extracted RNA was used as template for reverse transcription, followed by PCR and qPCR amplification detection. The results showed that RNA extracted by TransGen products had a higher concentration, better integrity, and could be better applied to downstream amplification detection.

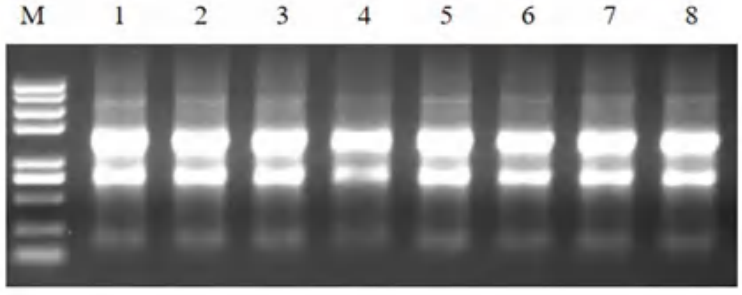


3. High stability

Automatic version (32×) stability test

Taking full account of the influence of well positions, 8 well positions were selected from column 1/7 of the 96-well plate for testing.

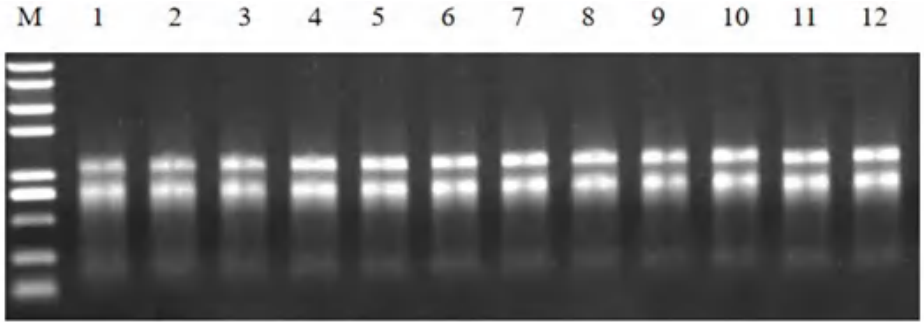
Well position	A260/A280	A260/A230	Nano concentration (ng/ul)
1	2.06	2.13	250.52
2	2.07	2.00	232.27
3	2.06	2.10	242.87
4	2.11	1.87	236.75
5	2.02	2.21	219.25
6	2.06	2.09	233.93
7	2.07	2.17	243.36
8	2.35	2.20	245.64
Average value			238.07
Standard deviation			9.78
Extraction precision			**0.041**



Automatic version (96×) stability test

Twelve well positions of the 96-well plate were randomly selected for extraction test.

Well position	A260/A280	A260/A230	Nano concentration(ng/ul)
1	1.976	1.802	180.04
2	1.984	1.915	186.01
3	1.993	1.952	187.65
4	1.996	2.025	184.72
5	1.974	1.896	183.25
6	1.976	1.894	187.80
7	1.985	1.917	184.72
8	1.993	1.949	184.55
9	1.997	1.966	182.32
10	1.992	1.940	186.06
11	1.978	1.877	181.11
12	1.990	1.896	186.73
Average value			184.58
Standard deviation			2.49
Extraction precision			**0.013**



PerfectStart® Universal Green qPCR SuperMix (AQ631)

Validation and Results

1. Fluorescence Curve Characteristics

Figure 1 shows a comparison of the amplification curves for AQ631, Control Reagent 1, Control Reagent 2, Control Reagent 3, and Control Reagent 4 on the Bio-Rad CFX96 instrument. The results indicate that the plateau phase height of AQ631 is higher than that of Control Reagent 3 and Control Reagent 4; slightly higher than Control Reagent 1; and slightly lower than Control Reagent 2 (based on a comprehensive assessment of other test data).

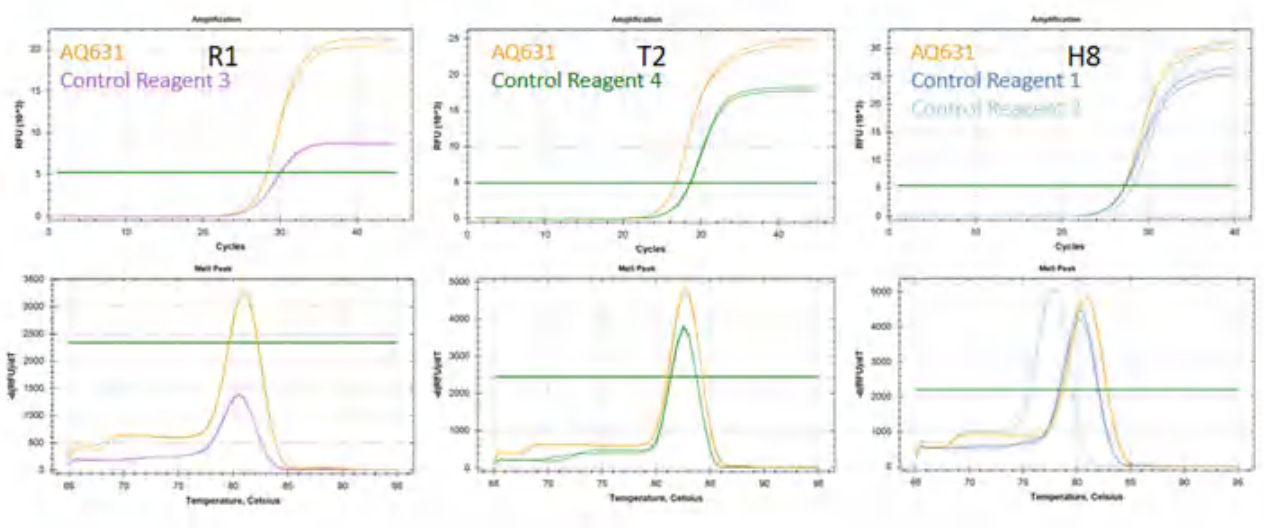


Figure 1. Comparison of amplification curves for AQ631 and control reagents on the Bio-Rad CFX96 instrument.

2. Ct Value

AQ631 and Control Reagent 1 were tested with cDNA templates from various species for 57 genes. Fifteen genes showed Ct value differences greater than 0.5 cycles. A summary of the differences is provided in Table 1, with red font indicating earlier Ct values for Control Reagent 1.

Table 1. Summary of Differentially Expressed Genes Between AQ631 and Control Reagent 1

Locus (Gene ID)	Ct Value Difference Between AQ631 and Control Reagent 1 (Red font indicates earlier Ct value for Control Reagent 1)	Locus (Gene ID)	Ct Value Difference Between AQ631 and Control Reagent 1 (Red font indicates earlier Ct value for Control Reagent 1)
T1	0.62	M9	-0.66
T3	0.55	W1	0.57
T7	-0.94	W8	1.18
A1	0.83	R10	-1.01
A8	0.51	R15	-0.58
H6	0.57	W9	-0.82
M1	-0.54	M11	-1.61
M2	0.54		

3. Amplification Efficiency

(1) Plasmid Standard Amplification

Four plasmid standards were used, each carrying the human ACTB gene, human TRPC6 gene, Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element (WPRE), and TSWV RNA S sequence. AQ631 and Control Reagent 1 were used to amplify 10-fold concentration gradients of the plasmid standards, and amplification efficiency and standard curve R^2 values were calculated. The amplification curves are shown in Figure 2. The amplification efficiency and standard curve R^2 values are shown in Table 2. The results indicate that AQ631 has better amplification efficiency and linearity, with some genes showing superior amplification efficiency or linearity compared to Control Reagent 1.

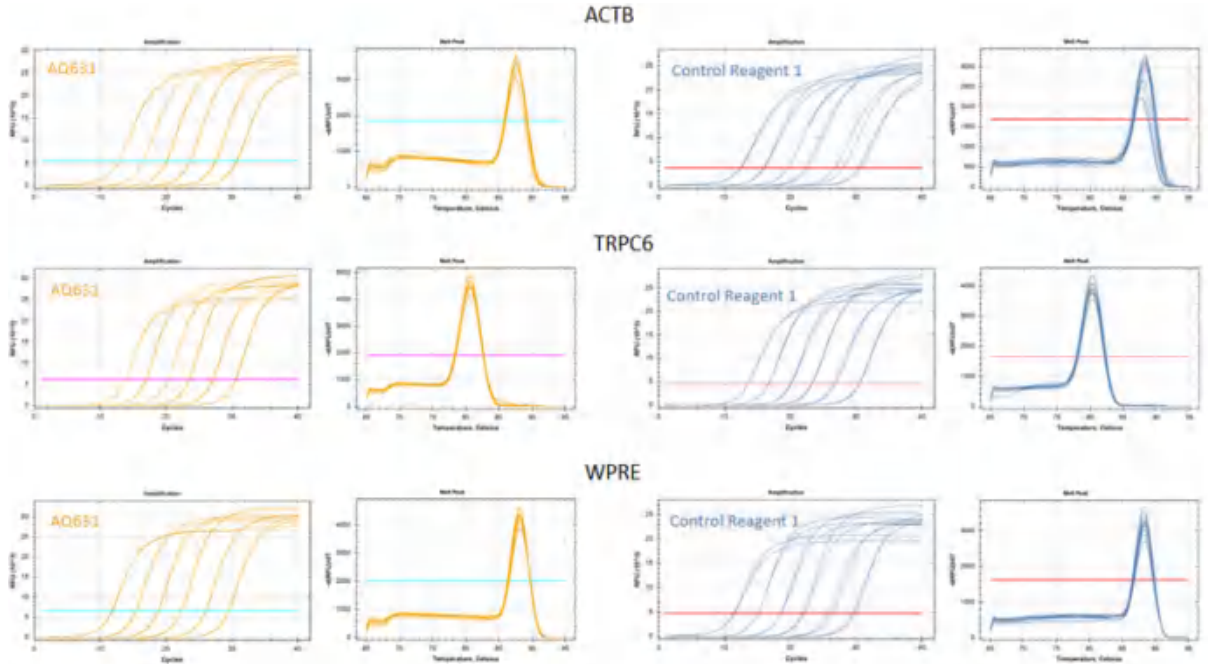


Figure 2. Amplification Curves of Plasmid Standards Using AQ631 and Control Reagents

Table 2. Amplification Efficiency and Standard Curve R^2 Values for Plasmid Standards Amplified by AQ631 and Control Reagents

ACTB	AQ631	Control Reagent 1	TRPC6	AQ631	Control Reagent 1
Amplification Efficiency	90.3%	88.0%	Amplification Efficiency	98.7%	98.1%
R^2	1.000	0.994	R^2	0.999	0.999
WPRE	AQ631	Control Reagent 1	TSWV RNA S	AQ631	Control Reagent 1
Amplification Efficiency	91.7%	88.2%	Amplification Efficiency	96.1%	97.0%
R^2	1.000	0.999	R^2	0.999	0.999

(2) Amplification of ADAR, GAPDH, MAP4, and BAZ2B Genes Using 2x Dilution Gradient of Human cDNA as Template

The ADAR, GAPDH, MAP4, and BAZ2B genes were amplified using a 2x dilution gradient of human cDNA as the template, with AQ631 compared to Control Reagents 1 and 2. The results demonstrate that AQ631 exhibited better amplification efficiency and linearity compared to the other reagents.

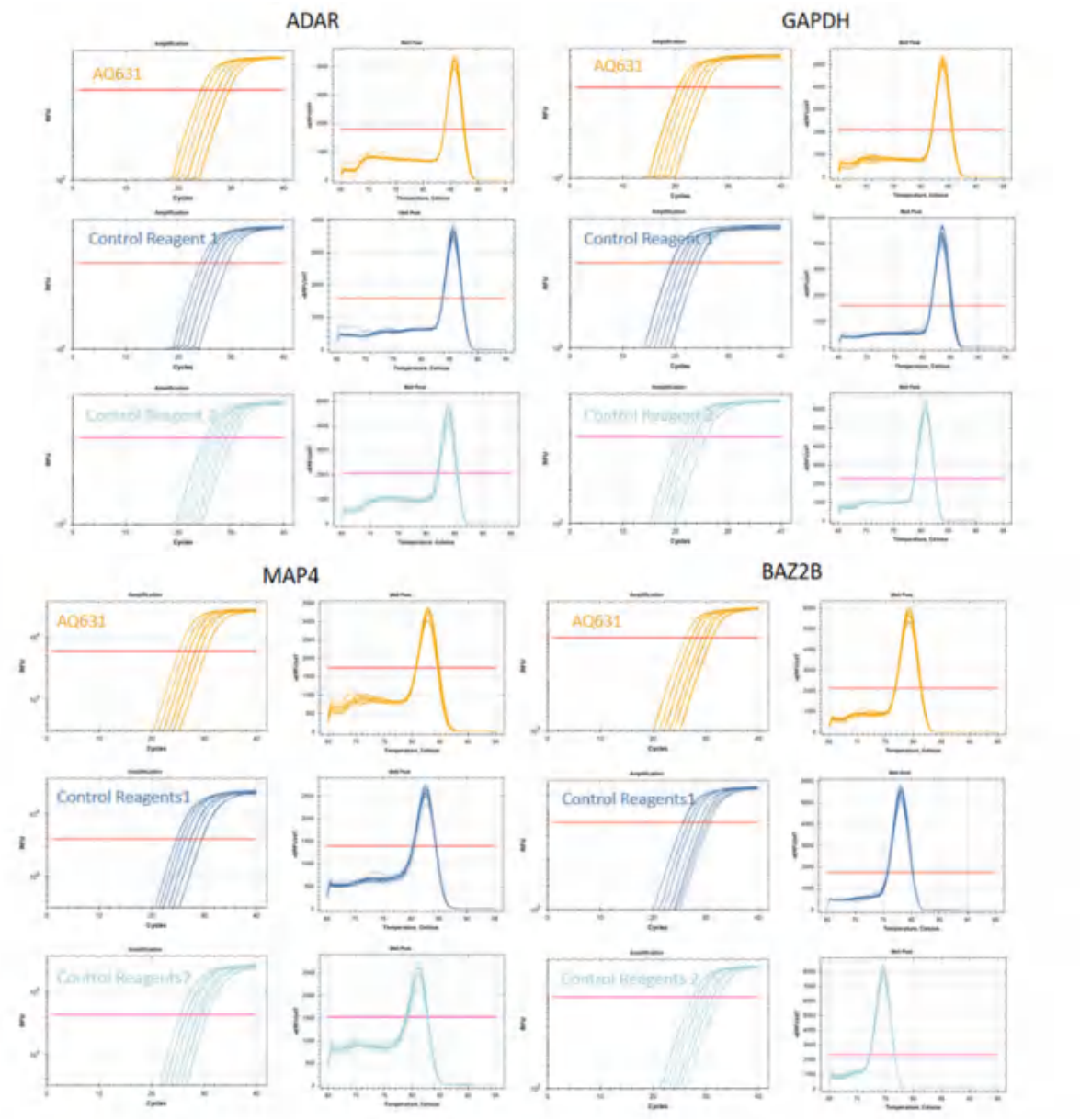


Figure 3. Amplification curve of 2-fold serially diluted cDNA using AQ631 and the control reagent.

Table 3. Amplification efficiency and standard curve R^2 of 2-fold serially diluted cDNA using AQ631 and the control reagent.

ADAR	AQ631	Control Reagent1	Control Reagent2	GAPDH	AQ631	Control Reagent1	Control Reagent2
Amplification Efficiency	92.2%	95.3%	97.7%	Amplification Efficiency	97.9%	98.1%	98.6%
R^2	0.998	0.997	0.996	R^2	0.998	0.998	0.999
MAP4	AQ631	Control Reagent 1	Control Reagent 2	BAZ2B	AQ631	Control Reagent 1	Control Reagent 2
Amplification Efficiency	97.6%	106.8%	97.1%	Amplification Efficiency	101.8%	102.5%	102.3%
R^2	0.999	0.994	0.995	R^2	0.998	0.993	0.996

4. Amplification Test of Genomic DNA from Plants Rich in Polysaccharides and Polyphenols

The cotton gene (G3), blueberry gene (B4), and grape gene (V3) were amplified using AQ631,

Control Reagent 1, and Control Reagent 2. The amplification results are shown in Figure 4. Both AQ631 and the two control reagents successfully amplified the genomic DNA from plants rich in polysaccharides and polyphenols.

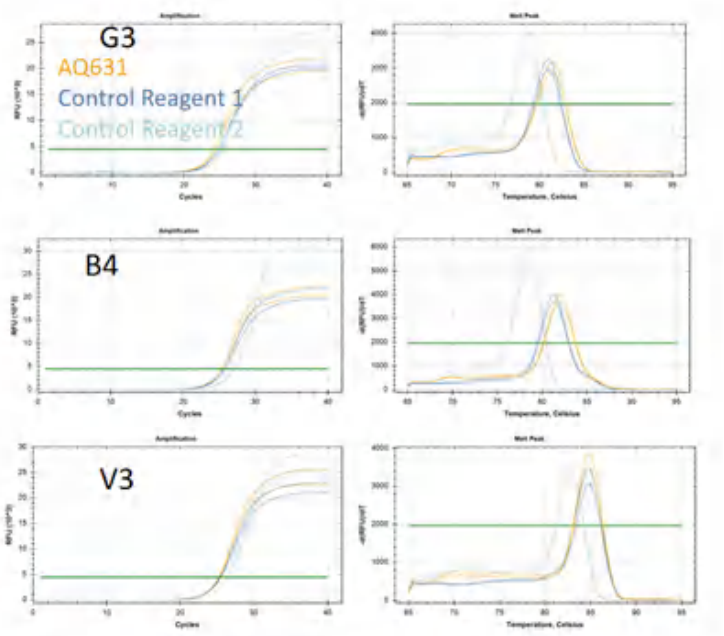


Figure 4. Amplification curve of genomic DNA templates from plants rich in polysaccharides and polyphenols using AQ631 and control reagents.

5. Instrument Compatibility Test

The human ACTB gene was amplified using AQ631 on the following qPCR instruments: Bio-Rad CFX96, Bioer FQD96A, TransGen TSQ96, ABI 7500, ABI StepOnePlus, and ABI QuantStudio7. The results are shown in Figure 5. The results indicate that AQ631 is compatible with multiple qPCR instrument models.

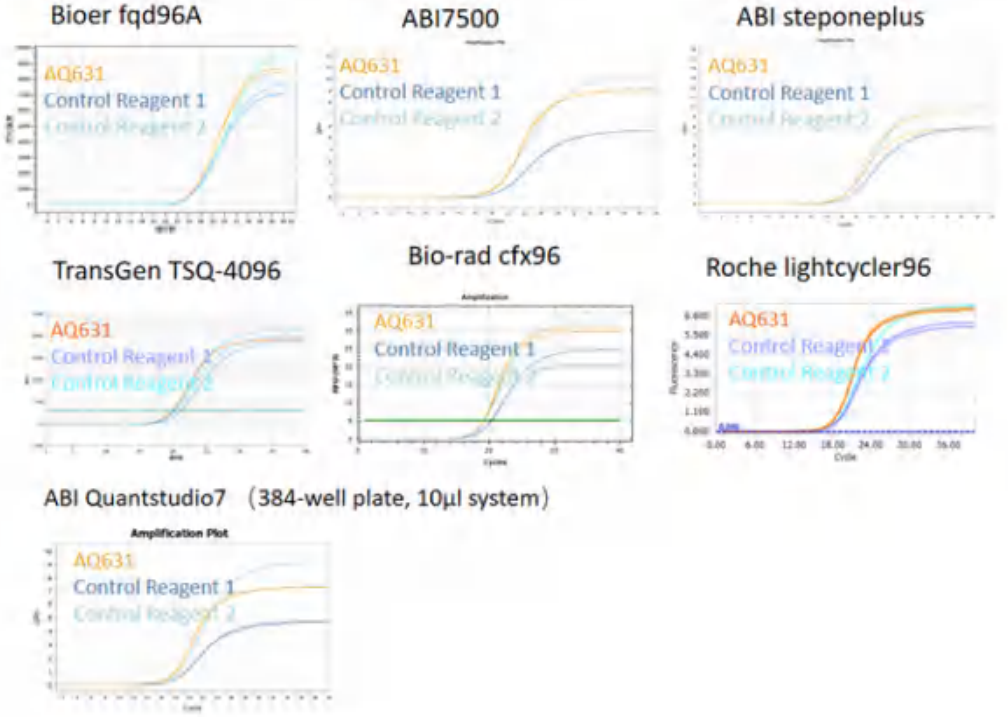


Figure 5. Instrument compatibility test results.

6. Stability Test of AQ631

(1) Reagent Freeze-Thaw Stability Test

The reagent was thawed at room temperature, mixed, and incubated at room temperature for 5 minutes with the cap open. Afterward, the cap was tightened and the reagent refrozen at -20°C for one freeze-thaw cycle. The reagent was subjected to 5 and 10 freeze-thaw cycles and tested for amplification of target genes (Gene IDs: R12, R13, R18, W6). The Ct values are shown in Table 4. The results indicate that the amplification performance of the reagent remained stable after 5 and 10 freeze-thaw cycles.

Table 4. Reagent Freeze-Thaw Test

	R12		R13		R18		W6	
	Ct value:	Ct value change:	Ct value:	Ct value change:	Ct value change:	Ct value change:	Ct value:	Ct value change:
Freeze-thaw 10 times	27.83	0.10	20.82	-0.12	22.39	0.27	33.46	0.05
Freeze-thaw 5 times	27.79	0.06	20.80	-0.13	22.31	0.20	33.81	0.41
Freeze-thaw 0 times	27.73	0.00	20.94	0.00	22.12	0.00	33.40	0.00

(2) Reagent Storage Stability at Elevated Temperature

The reagent was stored at 4°C and 37°C for 3 and 7 days, then tested for amplification of target genes (Gene IDs: H7, R13, R12) and compared with the reagent stored at -20°C. The Ct values are shown in Table 5. The results show that the reagent maintained stable amplification performance after being stored at 4°C and 37°C for 3 and 7 days.

Table 5. Reagent Storage Stability Test

	H7		R13		R12	
	Ct value:	Ct value change:	Ct value:	Ct value change:	Ct value change:	Ct value change:
4℃ 3 days	24.77	-0.04	21.20	0.07	28.18	0.06
4℃ 7 days	24.80	0.00	21.10	-0.03	28.16	0.05
37℃ 3 days	24.67	-0.13	21.05	-0.07	27.99	-0.13
37℃ 7 days	24.76	-0.04	21.10	-0.03	28.09	-0.03
-20℃ Control	24.80	0.00	21.13	0.00	28.12	0.00

Summary and Conclusion

The results of this performance report indicate that:

(1) AQ631 has strong fluorescence intensity during the plateau phase.

(2) The amplification performance and the standard curve's linearity are excellent, ensuring the accuracy of quantitative experiments.

(3) It can amplify genomic DNA from plants rich in polysaccharides and polyphenols.

(4) It is compatible with multiple qPCR instruments, including those that do not require ROX calibration, as well as those compatible with high ROX and low ROX.

(5) The reagent's performance remains stable under freeze-thaw and accelerated storage conditions.

pEASY®-Basic Seamless Cloning and Assembly Kit (CU201)

Description

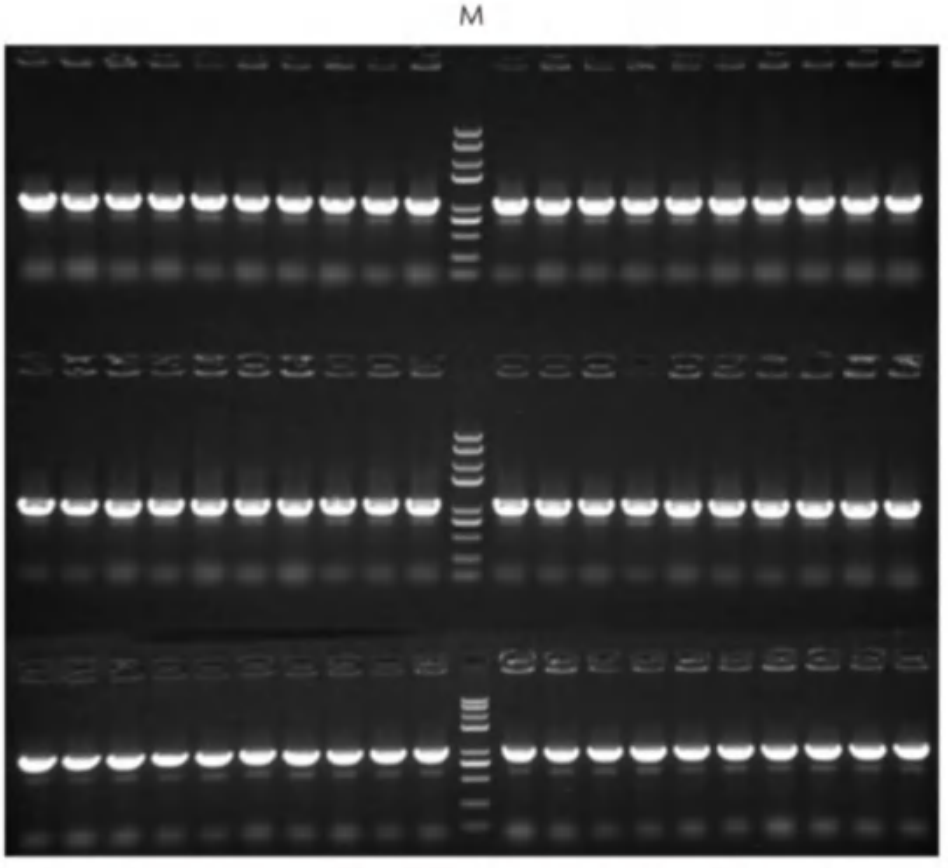
pEASY® -Basic Seamless Cloning and Assembly Kit takes advantage of proprietary recombinases and homologous recombination to achieve directional recombination of PCR inserts with 15-25bp overlapping ends into any linearized vector, enabling efficient and seamless assembly of 1-5 fragments.

Features

- Fast: 5~15 minutes.
- Simple: No restriction enzyme digestions.
- High efficiency: > 95% cloning positive rate.
- Seamless: No extra sequences introduced.

Single fragment ligation

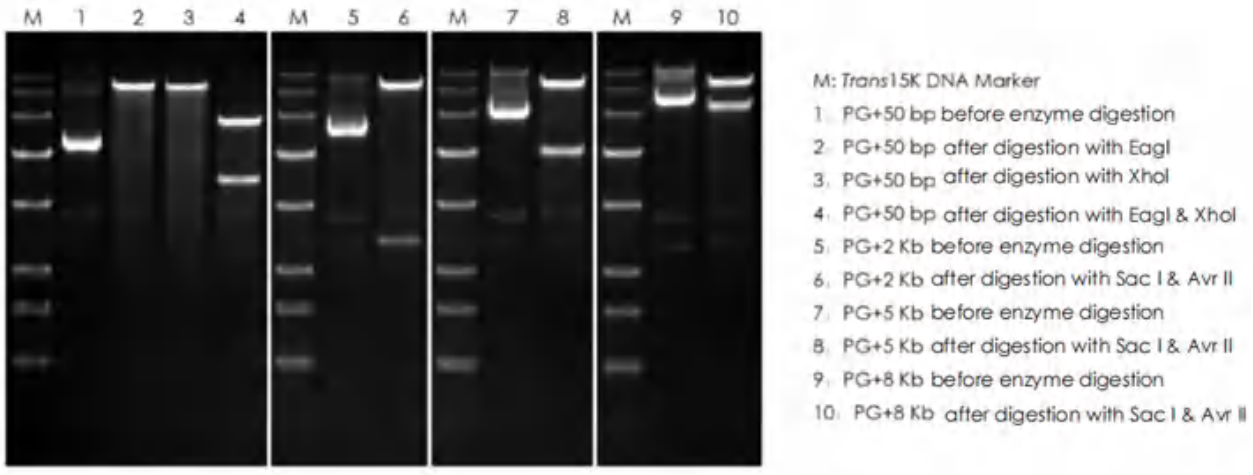
1 kb PCR fragment was inserted into the pUC19 vector, the results were analyzed by colony PCR. The results showed that the positive rate of the inserted fragment was 100%.



Colony PCR results of 60 clones
M: Trans2K® Plus II DNA Marker

Single fragment ligation of different sizes

PCR fragments of different sizes (50 bp, 2 kb, 5 kb, 8 kb) were inserted into the PG vector respectively, the ligation results were analyzed by enzyme digestion. The results showed that PCR fragments of different sizes were correctly ligated into the PG vector.



The single fragment ligation results were analyzed by enzyme digestion

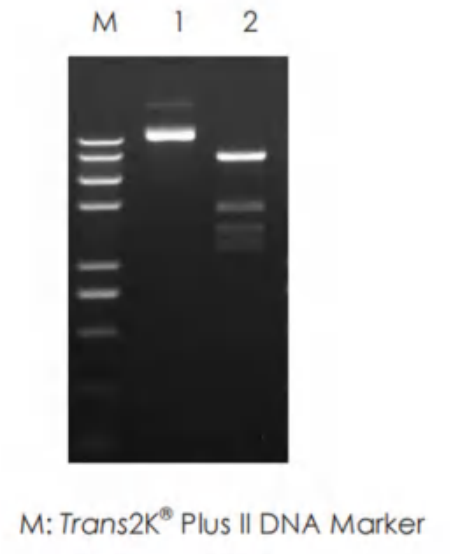
Multiple fragment ligation of different sizes

PCR fragments of different sizes (600 bp, 900 bp, 1200 bp, 1500 bp, with NdeI restriction sites designed at both ends of the fragment) were mixed and inserted into the pUC19 vector, the ligation results were analyzed by NdeI restriction enzyme digestion. The results showed that fragments of different sizes were correctly ligated into the pUC19 vector.

Multi-fragment ligation results were analyzed by enzyme digestion

Using TransGen products, 5 fragments of different sizes (0.5 kb, 1.2 kb, 1.4 kb, 1.8 kb, and 3.9 kb) were mixed and inserted into the vector, and the insertion effect was identified by enzyme digestion. The results showed that fragments of different sizes were correctly inserted into the vector

and multiple fragments were successfully ligated.



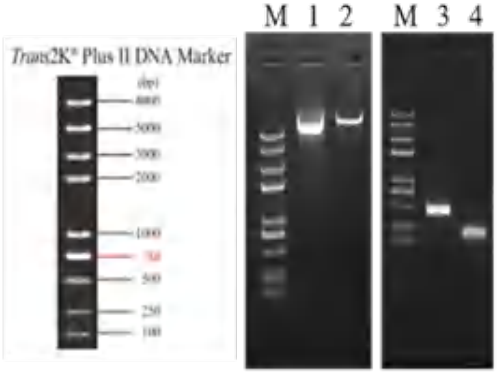
M: *Trans2K*® Plus II DNA Marker

1: Before enzyme digestion 2: After enzyme digestion

Customer feedback

A teacher from Peaking University

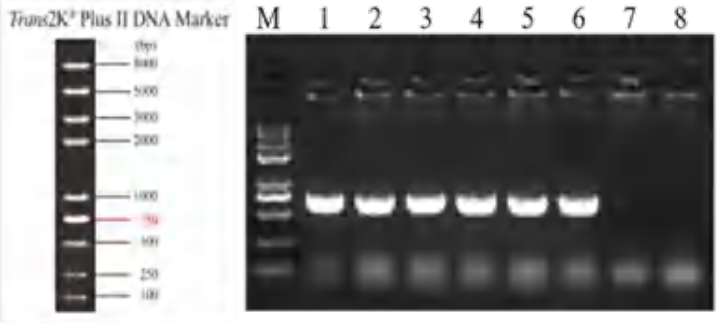
Successfully ligated a 15kb vector + 0.45kb fragment + 0.2kb fragment using the CU201 product.



Lane 1: Original plasmid

Lane 2: Linearized plasmid

Lane 3-4:Fragment 1/2



Lane 1-8:8 positive clones

TransIntro™ EL Transfection Reagent (FT201)

Description

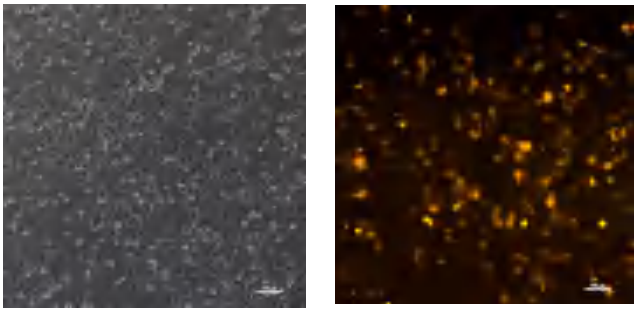
This product is a non-liposomal formulation designed to transfect DNA and small RNA into a wide variety of eukaryotic cell lines with high efficiency and low toxicity. Primary cells and other difficult-to-transfect cells can also be effectively transfected by this reagent.

Features

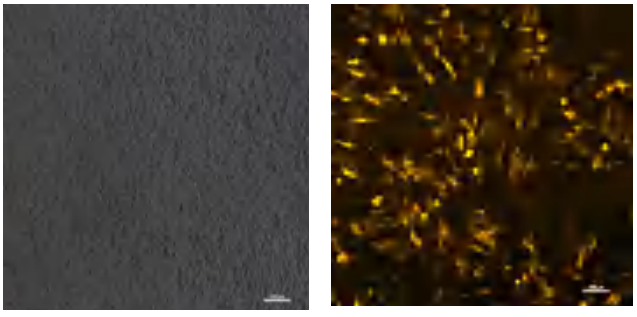
- High transfection efficiency: suitable for a wide range of cell lines, and can also obtain better results on primary cells and difficult-to-transfect cells, such as HepG2, CHO, K562 and Neuro-2a.
- Low cytotoxicity: Minimum effect on cellular physiological processes.
- Simple operation: No need to change the medium after transfection, tolerance with serum and antibiotics.
- Broad range of applications: Applicable for both DNA and small RNA.

Successfully transfected cell types with TransIntroTM EL Transfection Reagent

A549	COS-1	Hep G2	MEF	PANC-1
B16-F10	DLD-1	HL-60	MIA PaCa-2	PT67
BHK-21	HCT-116	K562	Neuro-2a	SGC-7901
BTC	HEK-293	L929	NIH/3T3	SH-SY5Y
C2C12CEF	HEK-293T	NRK	P815	Porcine epithelial granulosa cells
CHO	HeLa	Vero	U2OS	Bovine fibroblasts
CEF	COS-7	STO	MARC-145	Pilose antler stem cells
MCF-7				



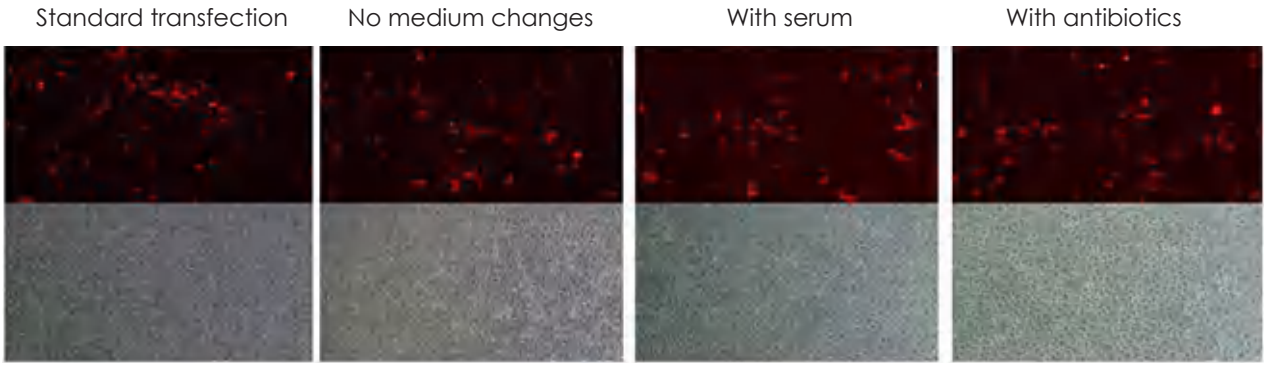
A549



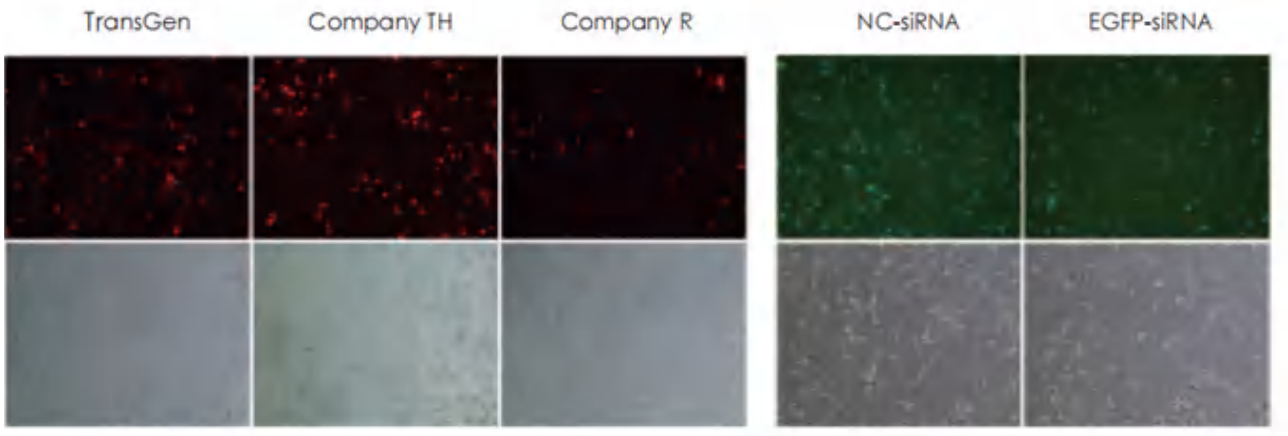
BHK-21

No need to change the medium after transfection, tolerance with serum and antibiotics

SGC-7901

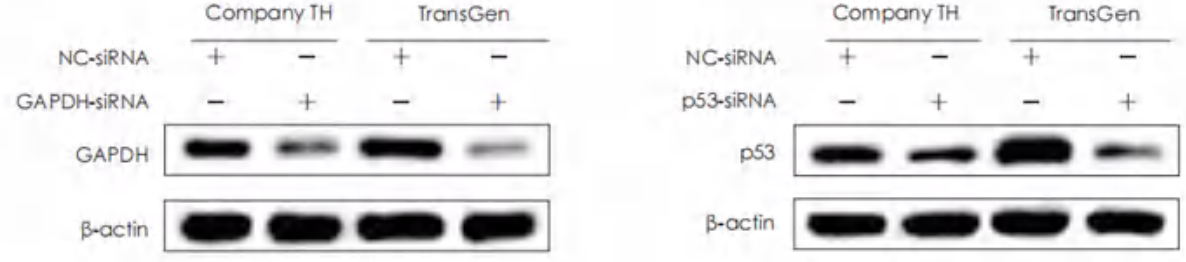


Comparation Data

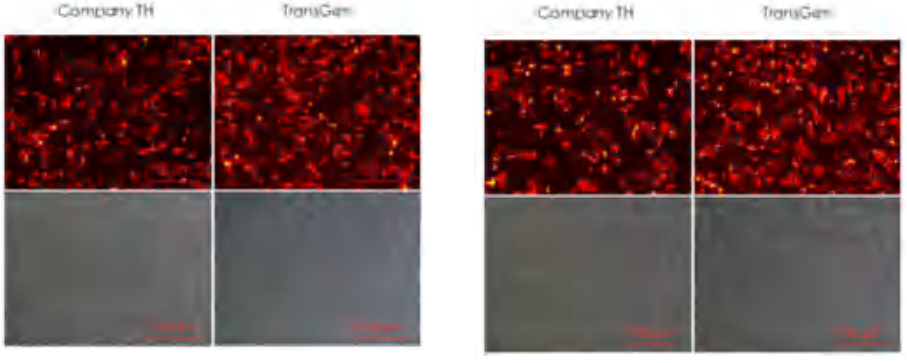


Transfected with DNA (HeLa)

Co-transfected with DNA and siRNA (HEK-293)



siRNA transfection effect with HEK-293 cells detected by Western Blot



Without serum

With serum

CHO-K1

TransDetect® Double-Luciferase Reporter Assay Kit (FR201)

• Product Overview

The *TransDetect*® Double Luciferase Reporter Assay Kit (FR201) is a highly sensitive dual-luciferase reporter gene detection kit. It contains high-purity Firefly luciferin and Coelenterazine substrates. The assay sequentially detects Firefly luciferase activity using luciferin as the substrate and subsequently quenches the signal before detecting Renilla luciferase activity using coelenterazine as the substrate within the same sample. This dual-luciferase assay is characterized by rapid detection, high sensitivity, a wide dynamic range, and minimal interference from endogenous cellular activities.

• Kit Performance Demonstration

293T cells were seeded into a 96-well plate and co-transfected with plasmids expressing Firefly luciferase and Renilla luciferase. After 24 hours, their expression levels were measured.

Compared to the competing product, Promega (E1910), FR201-V2 exhibited higher sensitivity and stronger luminescence for Firefly luciferase. The luminescence signal for Renilla luciferase was comparable to that of Promega. The half-life of the luminescence signal for Firefly luciferase was 10 minutes, while that of Renilla luciferase was 2 minutes.

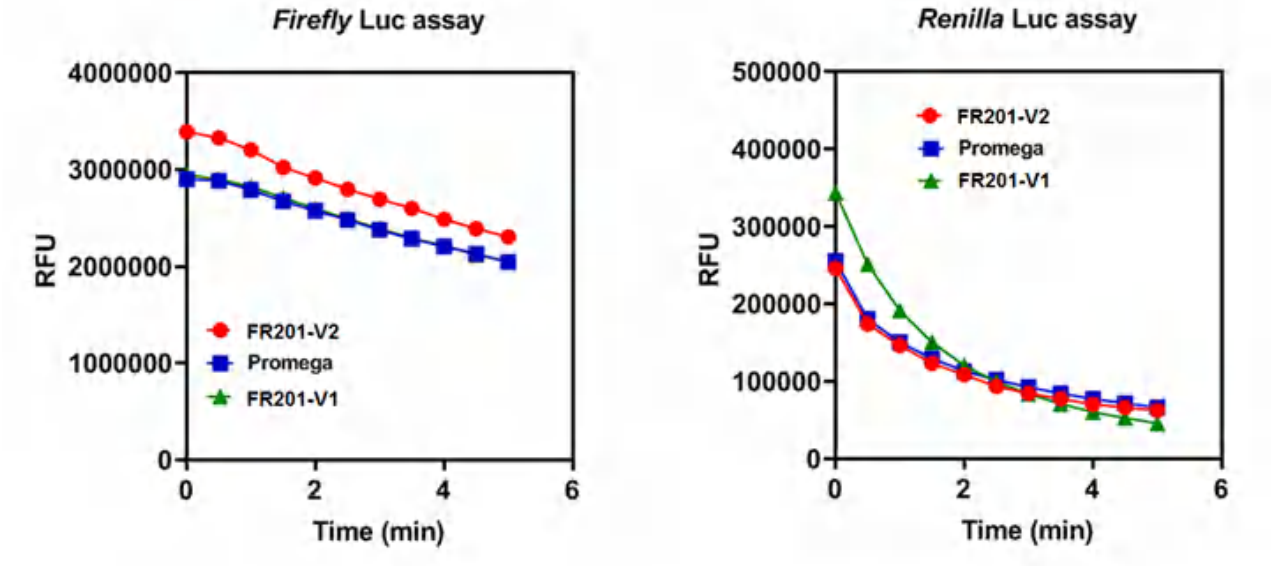


Figure 1. Comparison of Firefly and Renilla luciferase detection between FR201 and Promega

• Application Example: Data Comparison

The target gene was inserted into the 3' UTR of the psiCHECK2 plasmid (containing hRluc), resulting in a plasmid that expressed the target gene, Firefly luciferase, and Renilla luciferase. This plasmid was then co-transfected into cells with different concentrations of target gene siRNA, and the efficiency of siRNA-mediated gene silencing was assessed.

Table 1. Luminescence RLU readings for Firefly luciferase (Fluc)

FLUC	1 nM siRNA		5 nM siRNA		scramble siRNA	
Promega	31552	32242	32634	33148	31548	38412
	31965	42105	42508	52208	39510	40214
FR201-V2	33715	39318	43321	37678	41531	43521
	50604	50401	50621	55903	51861	49828

Table 2. Luminescence RLU readings for Renilla luciferase (Rluc)

RLUC	1 nM siRNA		5 nM siRNA		scramble siRNA	
Promega	9622	12726	7964	5825	19820	27409
	11982	9605	6596	7374	20166	30127
FR201-V2	12719	9721	7157	5300	28502	22205
	15120	11466	6433	6247	23286	22750

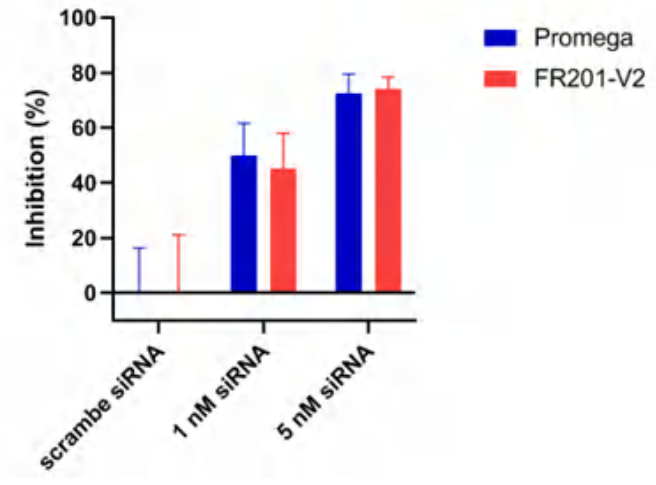


Figure 2. Inhibition rates of target gene expression by different concentrations of siRNA

TransDetect® Luminescent Cell Viability Detection Kit (FC401)

Methods and Results

1. Excellent detection sensitivity

Cell plates with gradient density were prepared for Jurkat cells using RPMI 1640 culture medium supplemented with 10% FBS. The cells were serially diluted in 2-fold or 5-fold ratios, resulting in cell suspensions of varying densities. 100 µl of cell suspension is pipette into each well of a 96-well plate, corresponding to gradients of 0, 5, 10, 50, 100, 500, 1000, 5000, 10000, and 50000 cells per well. After incubation at 37°C for 40 minutes, measurements were recorded. Upon reaching room temperature, an equal volume of FC401 or product from Company P was added to each well. In both large number (Figure 1A) and small number of cell/well conditions (Figure 1B), the fluorescence readings obtained from FC401 exceeded those obtained from Company P. Furthermore, a strong linear correlation between fluorescence readings and cell number was observed, with $R^2 > 0.99$. The ratio of fluorescence readings at 5 cells compared to those at 0 cells (culture medium only) was also higher for FC401, indicating greater sensitivity in detection (Figure 1C).

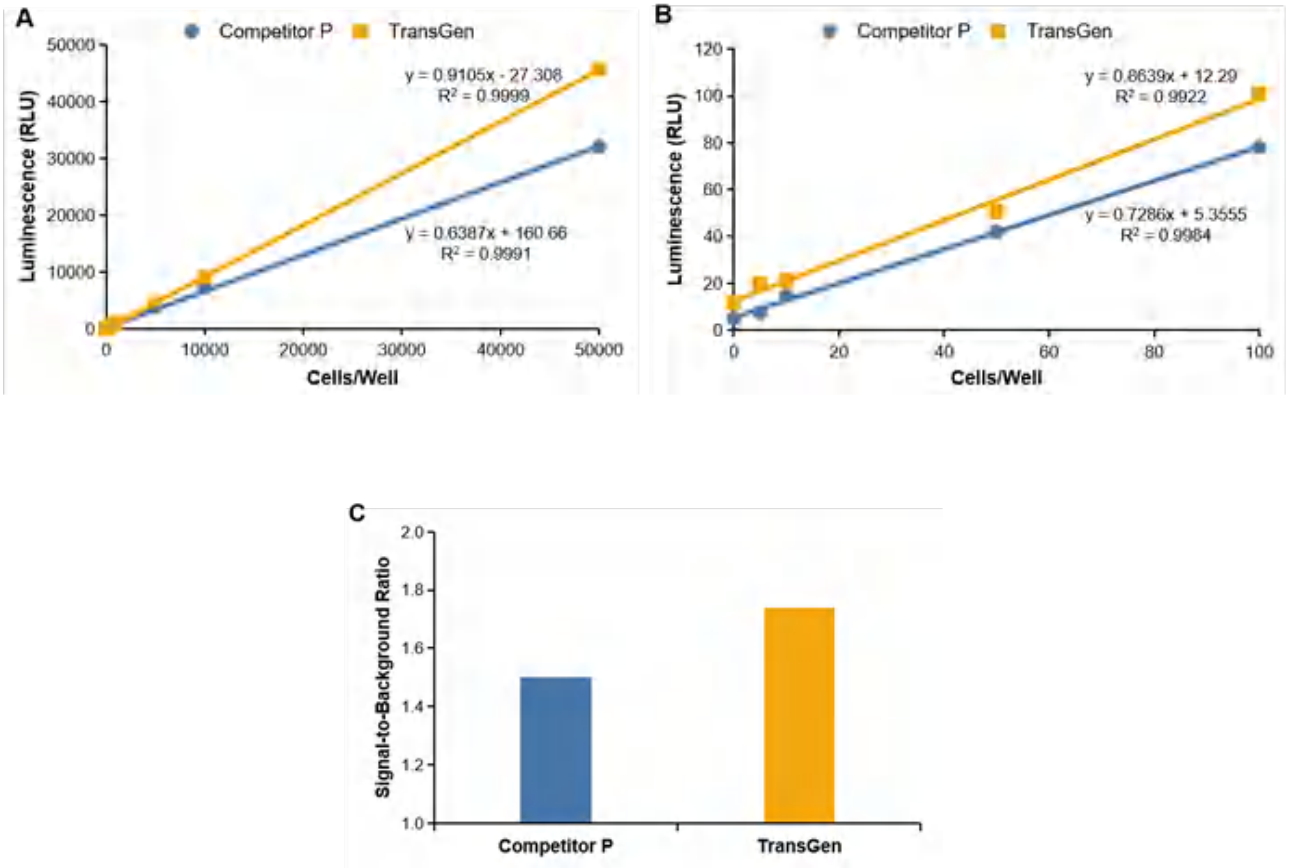


Figure 1. Comparison of the detection performance of the *TransDetect*® Luminescent Cell Viability Kit with product from Company P for Jurkat cell activity.

Figures A and B show a comparative analysis of the detection performance of FC401 and Company P across different cell numbers, specifically for 0-50,000 cells and 0-100 cells, respectively.

Figure C displays the ratio of fluorescence readings obtained at 5 cells to the background fluorescence readings for both FC401 and Company P.

2. Wide detection range of cell number

Based on the previous experiment, the number of cells increased to 100,000 for detection. The results are shown in Figure 2 below. When the cell count ranged from 0 to 100,000, the linear correlation between fluorescence readings and cell number remained stable, with $R^2 > 0.99$.

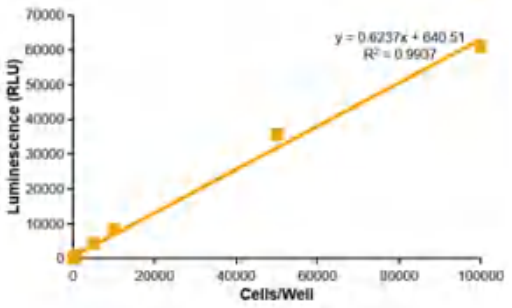


Figure 2. Detection results of Jurkat cell activity at 0-100,000 cells using FC401.

3. Stable detection signal reading

The operation of FC401 is more straightforward and efficient than other cell viability detection methods. It eliminates the need for washing and does not require the replacement or removal of the culture medium; the detection reagent can be added directly to the culture system, enabling a fast detection right after a 3-minute reaction. The "glowing" luminescent signal generated by FC401 is stable, with a half-life of up to 3 hours (Figure 3A). Within 30 minutes of the reaction, fluorescence readings decrease by no more than 5%, and by no more than 15% within 1 hour (Figure 3B). With these features, FC401 is suitable for both small sample detection and high-throughput screening on large scale.

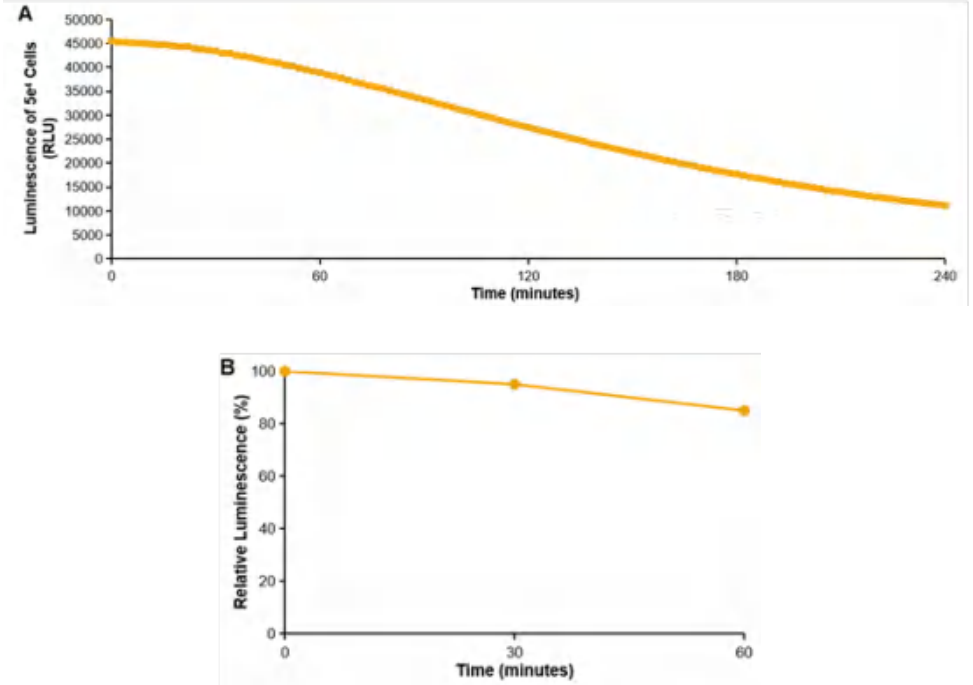


Figure 3. Measurement of the variation in fluorescence readings over time for 50,000 Jurkat cells using FC401.

Figure A displays the absolute fluorescence readings from 0 to 240 minutes. Figure B displays the ratios of fluorescence readings at 0, 30, and 60 minutes compared to the readings at 0 minutes.

4. Excellent product stability

FC401 can be stored at -20°C for long period of time. It maintains the ability to detect cell viability across different cell numbers when stored at 4°C for 14 days or at room temperature for 7 days. The results are shown in Figure 4, which give a strong linear correlation for both high and low cell counts.

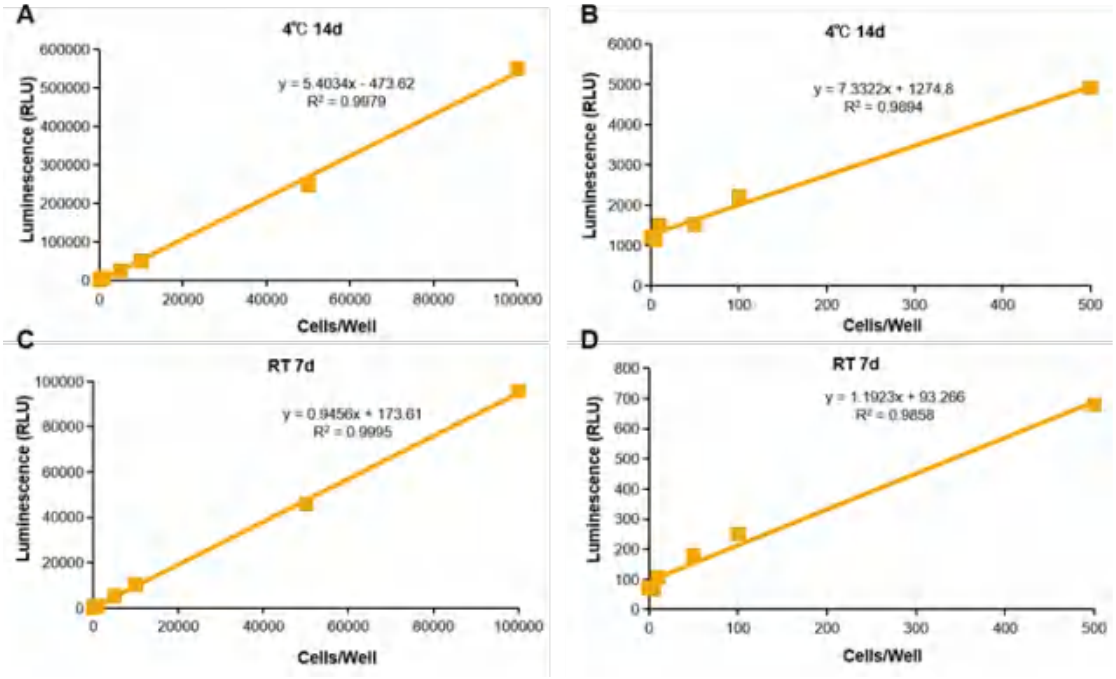


Figure 4. The detection results of cell viability across different cell counts under different storage conditions using FC401 is shown.

Figures A and B illustrate the results after storage at 4°C for 14 days, which gives a strong linear correlation between fluorescence readings and high cell counts (A) as well as low cell counts (B). Figures C and D illustrate the results after storage at room temperature for 7 days. Again, there is a strong linear correlation between fluorescence readings and high cell counts (C) as well as low cell counts (D).

5. Comparison with the CCK methodology.

The detection procedure for FC401 is fast and simple, allowing measurements to be made within 3 minutes after the reaction begin. Compared to traditional CCK methods for assessing cell viability, this product provides rapid experimental results and higher sensitivity. In figure 5, the results are generated using both methods to evaluate cell viability across different cell counts. For higher cell counts (0-100,000 cells, Figure 5A) and lower cell counts (0-100 cells, Figure 5B), the fluorescence readings from FC401 exhibit a stronger linear correlation, with $R^2 > 0.99$. Additionally, the fluorescence value corresponding to 5 cells is greater than the background fluorescence values (Figure 5E). In contrast, though the absorbance values (OD, measured after 1.5 hours using a microplate reader) detected by the CCK method (FC101) also show a linear relationship with higher cell counts (0-100,000 cells) and $R^2 > 0.99$ (Figure 5C), the linear relationship with low cell counts (0-100 cells) is poor (Figure 5D).

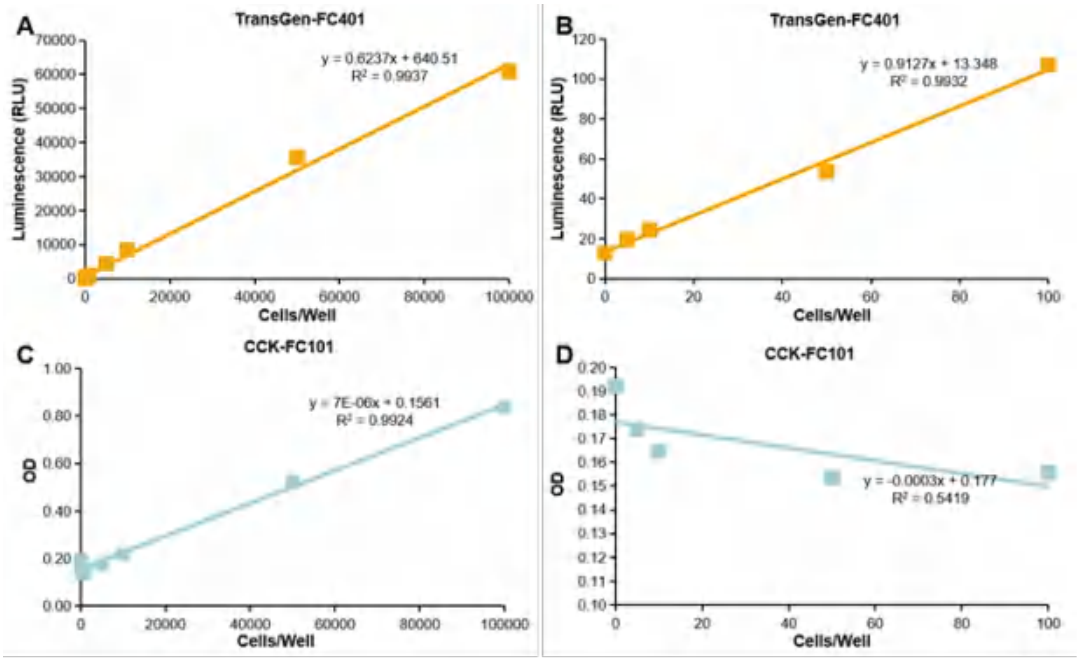


Figure 5. The detection results for 0-100,000 Jurkat cells activity using FC401 and CCK method. Figures A and B are the results generated by FC401, giving a linear correlation between fluorescence readings and cell counts of 0-100,000 and 0-100 cells, respectively. Figures C and D are the results generated by CCK method, giving a linear correlation between absorbance OD values and cell counts of 0-100,000 and 0-100 cells, respectively.

Sample Application

The bioactivity of recombinant human IL-2 cytokine was assessed using mouse CTLL-2 cells. The detection results using CCK method, FC401, and similar product from Company P are compared, as illustrated in Figure 6.

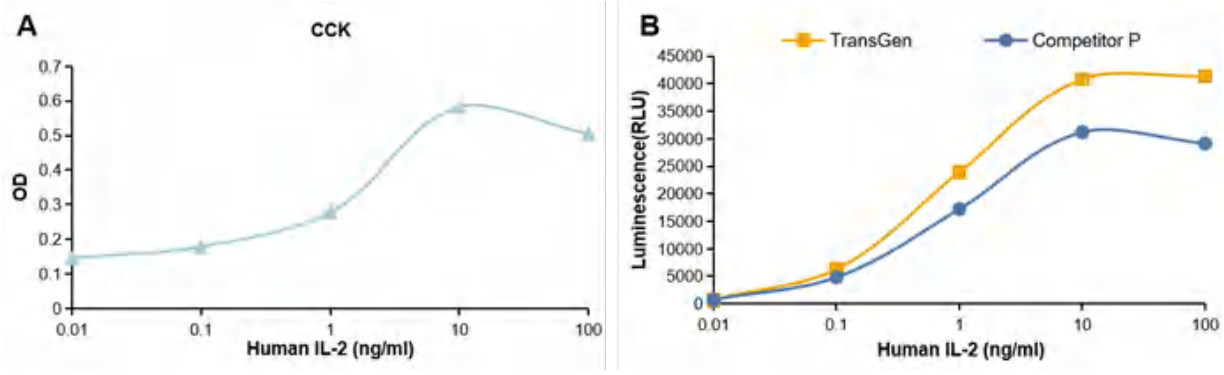


Figure 6. Assessment of the bioactivity of recombinant human IL-2 cytokine using CCK method and FC401. In figure A, cell viability detected by CCK method gives EC50=1.18 ng/ml. In figure B, cell viability detected by FC401 and Company P's product gives EC50(TranGen)=0.76ng/ml, EC50(Company P)=0.83 ng/ml.

Disclaimer: The actual readings obtained in this performance report may vary due to differences in cell types, detection instruments, and other factors; the data presented in the figures is for reference only.

ProteinSafe™ Protease Inhibitor Cocktail (100×)(DI111)

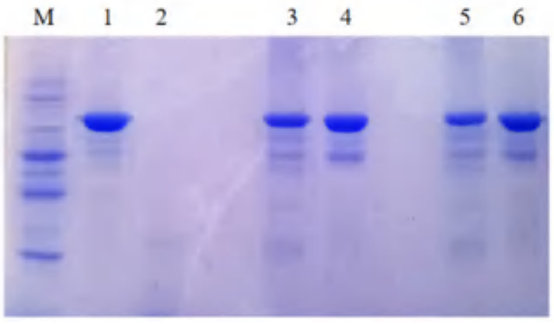
Description

ProteinSafeTM Protease Inhibitor Cocktail (100×) is a ready-to-use mixture of seven protease inhibitors (AEBSF, Aprotinin, Bestatin, E-64, Leupeptin, Pepstatin Aand EDTA) that has been optimized to protect proteins from being digested by endogenous proteases, including serine protease, cysteine protease, aminopeptidase, aspartic acid protease and metalloprotease. This cocktail is suitable for use in protein purification, Western Blot, Co-IP, ChIP, protein kinase activity assay, etc. It contains a separate component of EDTA which is incompatible with some downstream applications (i.e. protein assays, 2D electrophoresis, etc.). EDTA can be added to the cell lysis buffer if it is required as a metalloprotease inhibitor.

Features

- All-in-one format: a mixture of broad-spectrum protease inhibitors to prevent proteolytic degradation.
- Ready to use: no need to thaw and dissolve the cocktail, just use directly.
- Compatibility: compatible with most of protein lysis buffers composed of detergent and do not interfere with protein quantitation.

Data



Lane 1: BSA, incubation at 37℃ for 1 hour
Lane 2: BSA+Proteinase K (0.01μg), incubation at 37℃ for 1 hour
Lane 3, 5: BSA+Proteinase K (0.01μg)+ PIC+EDTA, incubation at 37℃ for 1 hour
Lane 4, 6: BSA+Proteinase K (0.01μg)+ PIC, incubation at 37℃ for 1 hour

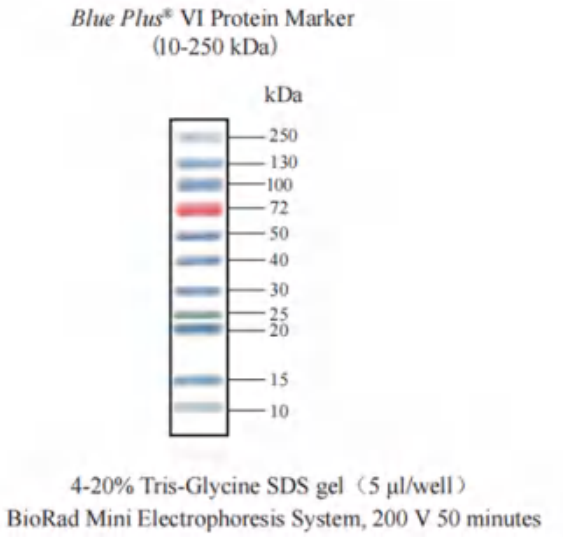
Some articles published using the ProteinSafeTM Protease Inhibitor Cocktail (100×) (DI111):

1 Wang Q, Shi B, Yang G, et al. Metabolomic profiling of Marek's disease virus infection in host cell based on untargeted LC-MS[J]. Front Microbiol, 2023.
2 Ma B, Zhou Y, Liu R, et al. Pigment epithelium-derived factor (PEDF) plays anti-inflammatory roles in the pathogenesis of dry eye disease[J]. The Ocular Surface, 2021.
3 Li J, Wang Y N, Xu B S, et al. Intellectual disability-associated gene ftsj1 is responsible for 2′-O-methylation of specific tRNAs[J]. EMBO reports, 2020.

Blue Plus® VI Protein Marker (10 kDa-250 kDa) (DM151)

Description

The product is prestained protein marker, composed of eleven prestained proteins ranging from 10 kDa to 250 kDa. The protein of 72 kDa band is covalently coupled to orange dye. The protein of 25 kDa band is covalently coupled to green dye. The other nine bands are covalently coupled to blue dye. This prestained protein marker is designed for monitoring the progress of SDS-polyacrylamide gel electrophoresis, for assessing transfer efficiency onto PVDF and NC membranes. Clear colored bands can be visible on PVDF or NC membranes. The orange and green bands make it easy to determine the direction of the transfer.



Data

Electrophoresis result

Transfer result

TRANSGEN BIOTECH CO., LTD.

Website www.transgenbiotech.com
Phone +86-10-57815030
Customer Service +86-400-898-0321
E-mail custserv@transgenbiotech.com