FUJIFILM
Value from Innovation

Wako

Test kits for residual DNA from host cells

01 For quantifying *E. coli*-derived genomic DNA by qPCR (probe-based method)
QCdetect™ Residual DNA Detection Kit for *E. coli*

02 For quantifying CHO cells-derived genomic DNA by qPCR (probe-based method)
QCdetect™ Residual DNA Detection Kit for CHO cells

03 For extracting trace amounts of host cell-derived DNA
DNA Extractor® Kit

Testing for residual DNA from host cells

A CHO cell or *Escherichia coli* is used as a host cell for expression of proteins, such as antibodies, in the manufacturing process of biopharmaceuticals. As DNA of these host cells may cause tumor formation, it is necessary to control the DNA as a process-related impurity.

Guidelines of the World Health Organization (WHO), the United States Food and Drug Administration (USFDA), and the European Pharmacopoeia (EP) state that the final amount of host residual DNA should be less than 100 pg/dose, or even as little as 10 ng/dose.

Fujifilm Wako offers a DNA extraction and purification kit, “DNA Extractor® Kit,” as a reagent for residual DNA testing. In addition, we have released residual DNA Detection Kit for *E. coli* and CHO cells.

Sample

DNA extraction and purification

DNA Extractor® Kit

DNA

qPCR

QCdetect™ Residual DNA Detection Kit for *E. coli*

QCdetect™ Residual DNA Detection Kit for CHO cells

DNA quantification

01 QCdetect™ Residual DNA Detection Kit for *E. coli*

This product is a kit for quantifying genomic DNA from *E. coli* by qPCR (probe method).

This kit is intended for use in the detection of residual host cell DNA in the quality control of biopharmaceuticals and in process development during manufacturing.

Features

- **Pre-mix formulation designed to be ready-to-use**
- Limit of detection : ≧ 0.03 pg/test
- Limit of quantitation : ≧ 0.3 pg/test
- Contains an internal control
- Calibration curve with high linearity
- Minimal inter-assay variability and high reproducibility
- Less susceptible to contaminants in samples

Kit contents

Components	Amount
1 x PCR Master Mix	2 x 1 mL
DNA Dilution Buffer (DDB)	1 x 10 mL
E. coli Control DNA	1 x 40 µL

Detection wavelength

- *E. coli* gDNA 520 nm（e.g., FAM）
- Internal Control 555 nm（e.g., HEX）

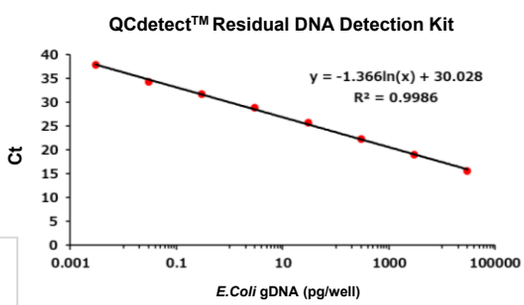
Note) Internal control is non-natural synthetic DNA.

Product No.	Product Name	Grade	Package Size
290-85301	QCdetect™ Residual DNA Detection Kit for *E. coli*	for Genetic Research	100 tests

Calibration curve

qPCR of 0.0003 pg to 30,000 pg of *E. coli* gDNA was performed using this kit to prepare a calibration curve (n = 3).

The calibration curve with high linearity was obtained over a wide concentration range from 0.03 pg to 30,000 pg.

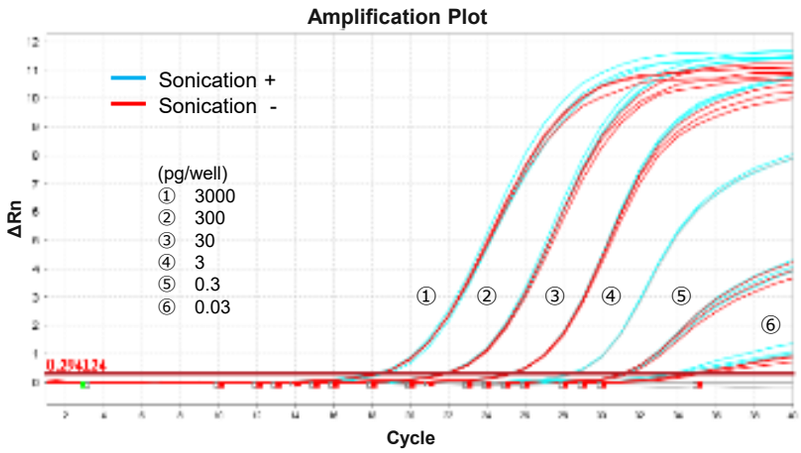


pg/well	0.0003	0.003	0.03	0.3	3	30	300	3000	30000
Ct	N.D	37.731	33.825	31.941	28.932	25.709	22.335	19.011	15.655
		38.012	34.866	31.684	28.875	25.675	22.414	18.976	15.393
		N.D	34.507	31.657	28.945	25.696	22.4	19.091	15.713
Average Ct	**N.D**	**37.872**	**34.399**	**31.761**	**28.917**	**25.693**	**22.383**	**19.026**	**15.587**
S.E	N.D	0.1989	0.5292	0.1566	0.0371	0.017	0.0418	0.0593	0.1707

Detection of fragmented DNA

E. coli gDNA was fragmented by sonication and detected by this kit to examine whether the fragmented *E. coli* gDNA could be detected.

Fragmented gDNA could be detected with the same sensitivity as intact gDNA.
A decrease in detection sensitivity was not observed, even for low concentrations of gDNA.

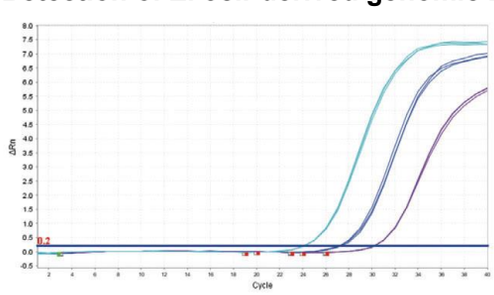


Example of use of this kit for samples containing high-concentration protein – combined with DNA Extractor® Kit –

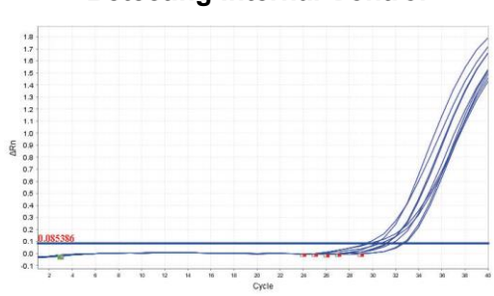
A sample containing 75 mg/mL human plasma-derived γ-globulin was spiked with *E. coli* gDNA at 0.1, 1, and 10 ng/mL, and DNA was extracted by DNA Extractor® Kit.
Then, the obtained DNA was quantified with QCdetect™ Residual DNA Detection kit for *E. coli*, and the spike recovery rate was obtained.

Amount of DNA spiked	0.1 ng/mL	1 ng/mL	10 ng/mL
Spiked DNA amount in the detection reaction (PCR)	1 pg	10 pg	100 pg
Recovery (mean)	96.9%	85.7%	86.9%
SD	0.013	0.67	1.4
CV%	1.3%	7.8%	1.6%

Detection of *E. coli*-derived genomic DNA



Detecting Internal Control

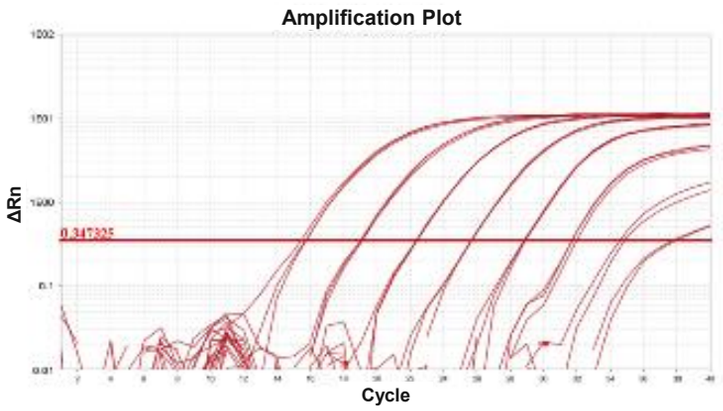


E. coli gDNA could be recovered with a high recovery rate, even when a high-protein sample was used.

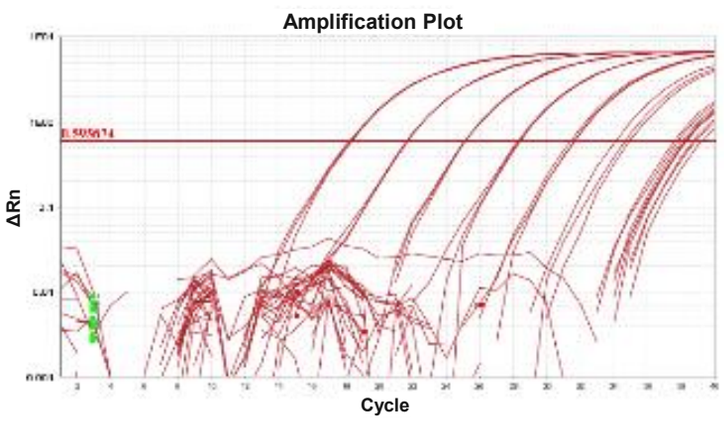
Comparison with other companies' *E. coli* genomic DNA detection kits

Detection performance

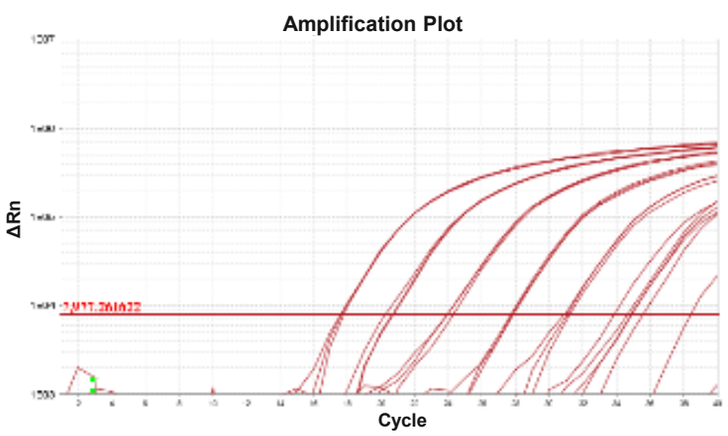
The amplification curves (in log scale) for *E. coli* gDNA in the range of 0.003–30,000 pg/well were compared among this kit and those from Company A and Company B.



Fujifilm Wako's product
Detection sensitivity : 0.03 pg/well



Company A's product
Detection sensitivity : 0.03 pg/well



Company B's product
Detection sensitivity : 0.3 pg/well
No IC used

Compared with other products, Fujifilm Wako's product demonstrated comparable or superior performance in terms of both detection sensitivity and reproducibility of amplification curves.

02 QCdetect™ Residual DNA Detection Kit for CHO cells

This product is a kit for quantifying genomic DNA from CHO cells by qPCR (probe method).

This kit is intended for use in the detection of residual DNA in antibody therapeutics and their manufacturing processes.

Features

- **Pre-mix formulation designed to be ready-to-use**
- Limit of detection : ≧ 0.0003 pg/test
- Limit of quantitation : ≧ 0.003 pg/test
- Contains an internal control
- Calibration curve with high linearity
- Minimal inter-assay variability and high reproducibility
- Less susceptible to contaminants in samples

Kit contents

Components	Amount
1 x PCR Master Mix	2 x 1 mL
DNA Dilution Buffer (DDB)	1 x 10 mL
CHO Control DNA	1 x 40 µL

Detection wavelength

- CHO gDNA 520 nm (e.g., FAM)
- Internal Control 554 nm (e.g., HEX)

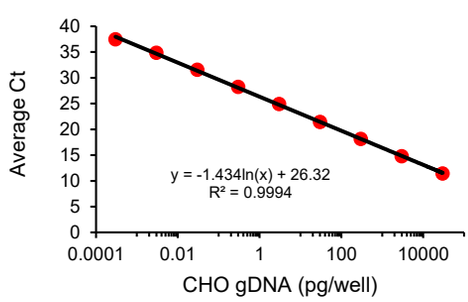
Note) Internal control is non-natural synthetic DNA.

Product No.	Product Name	Grade	Package Size
294-85201	QCdetect™ Residual DNA Detection Kit for CHO cells	for Genetic Research	100 tests

Calibration curve

qPCR of 0.0003 pg to 30,000 pg of CHO gDNA was performed using this kit to prepare a calibration curve (n = 3).

A calibration curve with very high linearity was obtained over a wide concentration range from 0.0003 pg to 30,000 pg.

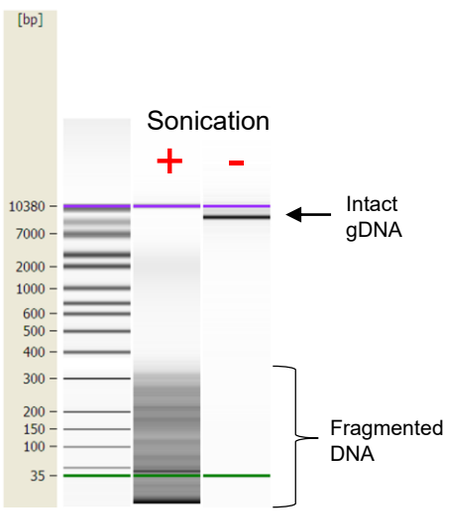


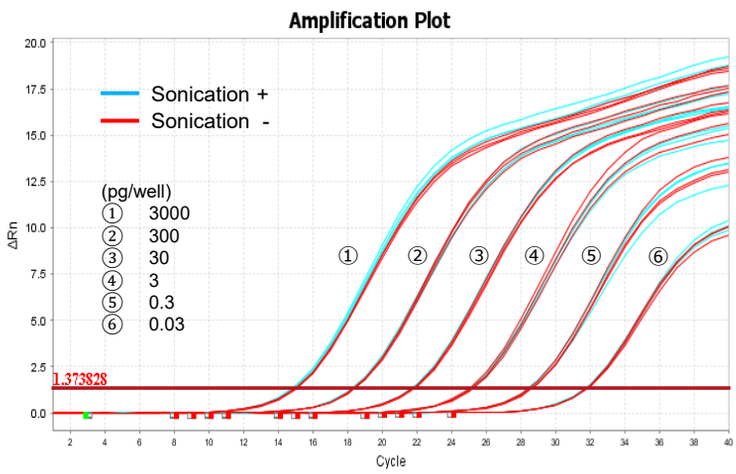
pg/well	0.0003	0.003	0.03	0.3	3	30	300	3000	30000
Ct	37.639	34.822	31.546	28.117	24.809	21.337	18.079	14.726	11.506
	37.107	34.981	31.506	28.324	24.906	21.416	18.084	14.744	11.467
	37.642	34.689	31.595	28.267	24.987	21.533	18.225	14.807	11.269
Average Ct	**37.462**	**34.830**	**31.549**	**28.236**	**24.901**	**21.428**	**18.129**	**14.759**	**11.414**
S.E	0.30806	0.14652	0.04455	0.10713	0.08904	0.09853	0.0831	0.0424	0.12685

Detection of DNA fragments

CHO gDNA was fragmented by sonication and detected by this kit to examine whether the fragmented CHO gDNA could be detected.

Fragmented gDNA could be detected with the same sensitivity as intact gDNA.
A decrease in detection sensitivity was not observed, even for low concentrations of gDNA.





Example of use for a high-protein sample - combined with DNA Extractor® Kit -

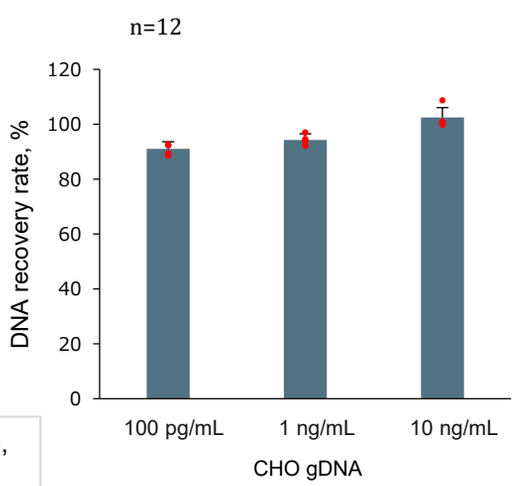
CHO gDNA was spiked to a sample containing high concentration of γ-globulin, and DNA was extracted by DNA Extractor® Kit.
The obtained DNA was quantified with QCdetect™ Residual DNA Detection Kit for CHO cells, and the spike recovery rate was obtained.

▶ Sample composition

- 20 mg/mL γ-globulin
- 3% Mannitol
- 2% Sucrose
- 10 mM L-Arginine
- 0.01% Tween20

▶ Concentration of spiked CHO gDNA

10 ng/mL
1 ng/mL
100 pg/mL

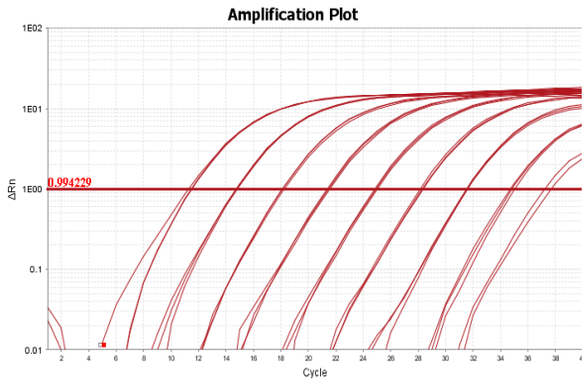


CHO gDNA could be recovered with a high recovery rate, even when a high-protein sample was used.

Comparison with other companies' CHO cells genomic DNA detection kits

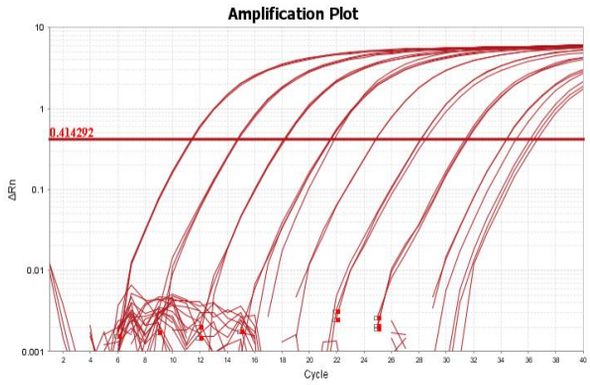
Detection performance

The amplification curves (in log scale) for CHO cells gDNA in the range of 0.00003–30,000 pg/well were compared among this kit and those from Company A and Company B.



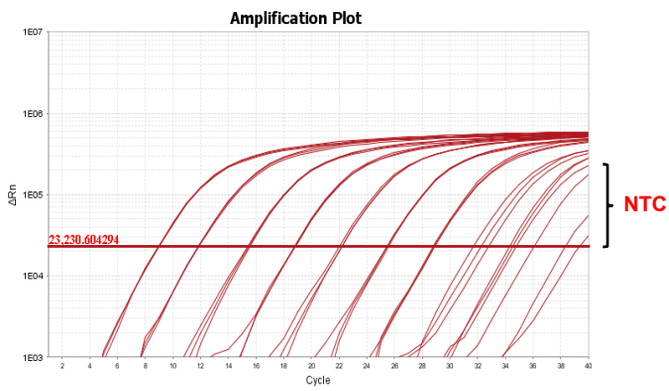
Fujifilm Wako's product

Detection sensitivity : 0.0003 pg/well



Company A's product

Detection sensitivity : 0.0003 pg/well



Company B's product

NTC (No-template control) amplification

Compared with other products, Fujifilm Wako's product demonstrated comparable or superior performance in terms of both detection sensitivity and reproducibility of amplification curves.

FAQ about QCdetect™ Residual DNA Detection Kit

Q.	A.
What qPCR method is used in this kit?	TaqMan Probe method
What is used as the internal control?	A synthetic sequence based on primer design.
Does the QCdetect™ Residual DNA Detection Kit for *E. coli* amplify genomic DNA other than that derived from *E. coli*?	No amplification has been observed with human, yeast, or CHO DNA.
Does the QCdetect™ Residual DNA Detection Kit for CHO cells amplify genomic DNA other than that derived from CHO cells?	No amplification has been observed with human, *E. coli*, or yeast DNA.
Various CHO cell lines exist— which ones can be detected?	Detection performance has been confirmed for CHO-K1, CHO-S, ExpiCHO, USP CHO Control, and CHO-Spica.
Are the primer/probe sequences the same as those described in the USP?	Yes, the same sequences as those in the USP are used.

03 DNA Extractor® Kit

The kit for extracting residual DNA from host cells by sodium iodide method.
The extracted DNA can be quantified by qPCR.
This kit is intended for use in testing and monitoring host cell–derived DNA from CHO cells, *E. coli*, yeast, etc., in combination with the QCdetect™ Residual DNA Detection Kit.

- High recovery of trace amounts of DNA
- No need for tube replacement
  (all the processes can be completed in the same tube)
- Only 60-90 minutes needed from start to completion of extraction
- Protocol available for samples containing high concentrations of protein
- Sodium iodide method* is adopted

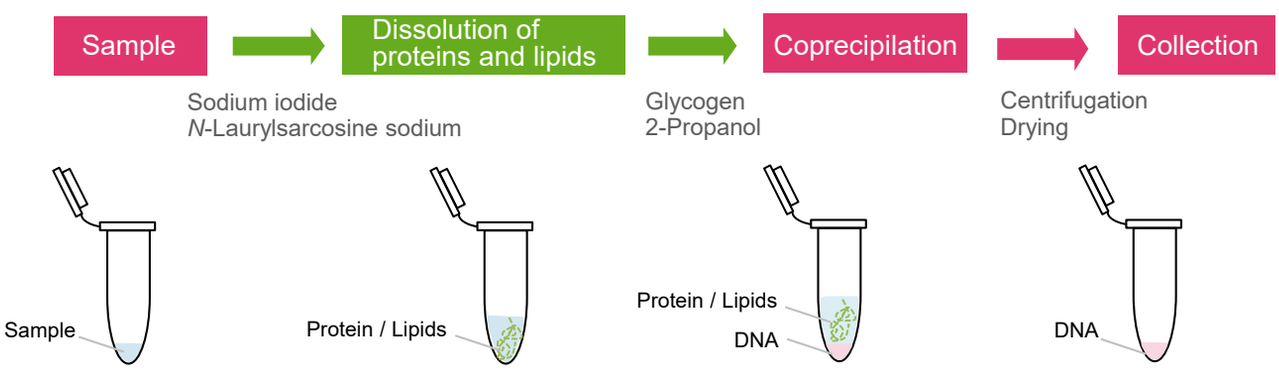
* The sodium iodide method is a residual DNA extraction technique that is described in the United States Pharmacopeia (USP) 42-NF37, <509> “Residual DNA Testing.”

Kit contents

Components	Amount
Sodium Iodide Solution	26 mL x 1
Sodium *N*-Lauryl Sarcosinate Solution	1.2 mL x 1
Washing Solution (A)	42 mL x 1
Washing Solution (B)	40 mL x 2
Glycogen Solution	0.1 mL x 1

Principle

1. Sodium iodide, a chaotropic ion, and sodium laurylsarcosine are added to a sample to solubilize proteins and lipids.
2. Glycogen and then 2-propanol are added to the sample, and DNA is coprecipitated with glycogen.
3. A DNA pellet is collected.



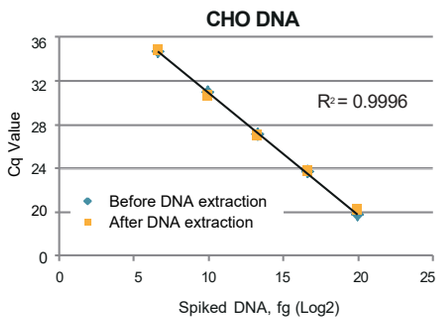
Product No.	Product Name	Grade	Package Size
295-50201	DNA Extractor® Kit	for Genetic Research	50 tests

Example of spike recovery test

A spike recovery test was performed with gDNA from 3 types of cells : CHO, *E.coli*, and *Pichia pastoris*.

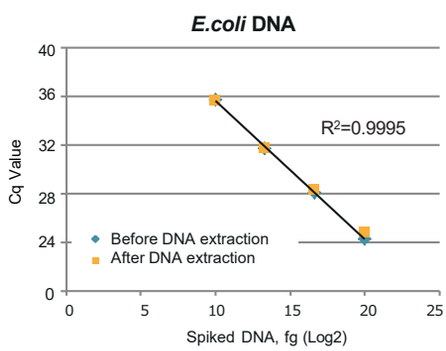
▶ CHO gDNA

gDNA	CHO	
Spike (fg)	Detection (fg)	Recovery (%)
100	100	100
1,000	979	98
10,000	10,831	108
100,000	90,031	90
1,000,000	1,045,186	105



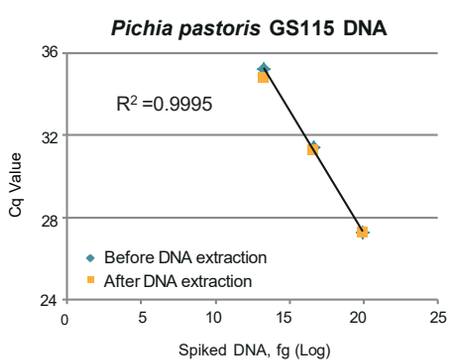
▶ *E.coli* gDNA

gDNA	*E.coli*	
Spike (fg)	Detection (fg)	Recovery (%)
1,000	939	94
10,000	11,004	110
100,000	100,009	100
1,000,000	969,911	97



▶ *Pichia pastoris* GS115 gDNA

gDNA	*Pichia pastoris* GS115	
Spike (fg)	Detection (fg)	Recovery (%)
10,000	10,316	103
100,000	94,045	94
1,000,000	1,031,630	103



A high DNA recovery was observed under all conditions. High linearity was observed between the amount of spiked DNA and Cq value.

Spike recovery test of CHO gDNA using culture supernatant

The spike recovery rate of CHO-derived DNA was determined using culture supernatant.

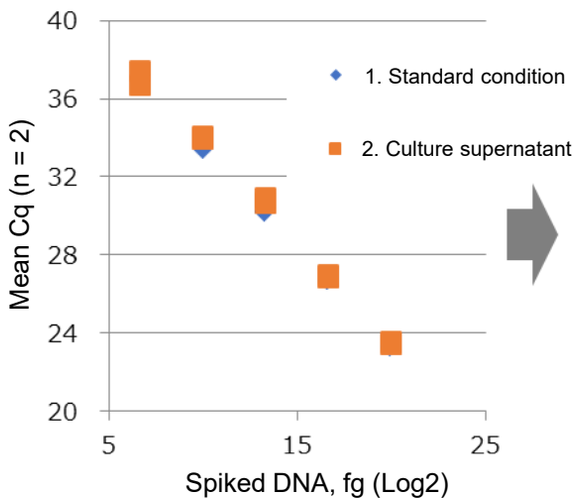
Methods:

CHO-derived DNA (10 fg to 1 ng) was spiked to culture supernatant of PANC-1 cells and the DNA was extracted with this kit. qPCR of the extracted DNA was performed and Cq value was measured. Separately, qPCR was carried out for purified water spiked with CHO-derived DNA without DNA extraction, and Cq value was measured. This was used as a standard condition.

A calibration curve was prepared under the standard condition, and DNA recovery rate was calculated.

Sample:

1. Standard condition: purified water spiked with CHO-derived DNA (without DNA extraction)
2. Culture supernatant condition: DNA extracted from PANC-1 cell culture supernatant spiked with CHO-derived DNA using this kit



Amount of spiked DNA and mean Cq
1. Standard condition: R^2= 0.9998
2. Culture supernatant: R^2= 0.9982

	Culture supernatant	
Spiked amount (fg)	Volume recovered (fg)	Recovery rate (%)
0	ND	
10		
100	93	**93**
1,000	741	**74**
10,000	6,333	**63**
100,000	86,703	**87**
1,000,000	874,502	**87**

A calibration curve was prepared using mean Cq values obtained under the standard condition, and DNA recovery rate was calculated from the mean Cq value obtained under the culture supernatant condition.

DNA in culture supernatant could be recovered in high yield within the range from 100 fg to 1 ng of spiked DNA.

Related Product

DNA Extractor® Kit Series

The DNA Extractor® Kit series is a DNA extraction and purification kit based on the sodium iodide method. Because DNA is not lost through adsorption to solid supports, and all procedures are completed within a single tube, exceptionally high recovery rates are achieved.
No hazardous reagents such as phenol or chloroform are used.

List of DNA Extractor® Kit series using the sodium iodide method and residual DNA detection kit

Sample	Product Name	Features
Viral DNA from serum Residual host cell-derived DNA from biological products	DNA Extractor® Kit	Extract trace amount of DNA from samples
Whole blood (human, bovine, equine), cultured cells, tissues	DNA Extractor® WB Kit	Extract high-molecular DNA
Genomic DNA in serum/plasma	DNA Extractor® SP Kit	Remove blood-derived lipids
Body hairs, nails, blood stains, saliva stain (forensic samples)	DNA Extractor® FM Kit	DNA extraction from trace amount of samples

Product No.	Product Name	Grade	Package Size
291-50502	DNA Extractor® WB Kit	for Whole Blood DNA Extraction	50 tests
296-60501	DNA Extractor® SP Kit	for Genetic Research	50 tests
295-58501	DNA Extractor® FM Kit	-	50 tests

Japan
FUJIFILM Wako Pure Chemical Corporation
1-2, Doshomachi 3-Chome, Chuo-ku, Osaka 540-8605, Japan
ffwk-cservise@fujifilm.com
labchem-wako.fujifilm.com

The Americas
FUJIFILM Biosciences
2501 Pullman Street, Santa Ana, CA 92705, USA
supportfisilssupport@fujifilm.com
fujifilmbiosciences.fujifilm.com

Europe, Middle East, and Africa
FUJIFILM Wako Chemicals Europe GmbH
Fuggerstr 12, 41468 Neuss, Germany
labchem_wkeu@fujifilm.com
labchem-wako.fujifilm.com

Chinese Mainland
FUJIFILM Wako (Guangzhou) Trading Corporation
Room 3002, 3003, 3011, 30/F., Dong Shan Plaza,
69 Xian Lie Middle Road, Yuexiu District, Guangzhou, 510095, China
wkgz.info@fujifilm.com
labchem.fujifilm-wako.com.cn

Hong Kong SAR
FUJIFILM Wako Chemicals (Hong Kong) Limited
Units 9-12 and 15-18, Level 28, Tower 1, The Millennity,
98 How Ming Street, Kwun Tong, Kowloon, Hong kong
wkhk.info@fujifilm.com
labchem.fujifilm-wako.com.cn

Other Areas
fujifilm.com/ffwk/en/about/partners/labchem