FUJIFILM
Value from Innovation

Wako

Test kits for residual DNA from host cells

01 A kit for quantifying genomic DNA from *E. coli* by qPCR (probe method)

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

02 A kit for quantifying genomic DNA from CHO cells by qPCR (probe method)

QCdetect™ Residual DNA Detection Kit for CHO cells

03 A kit for extracting DNA derived from host cells or serum

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 antibodies or proteins 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.

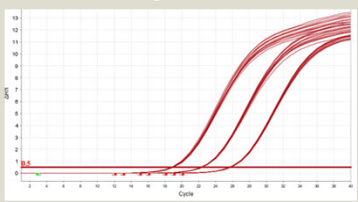
Fujifilm Wako Pure Chemical Corporation offers a DNA extraction and purification kit, "DNA Extractor® Kit," as a reagent for residual DNA testing. In addition, we have newly released residual DNA Detection Kit for *E. coli* and CHO cells.

Sample → DNA → DNA quantification

DNA extraction and purification

DNA Extractor® Kit

qPCR



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

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).
It can be used for residual DNA testing for biopharmaceuticals and their manufacturing processes.

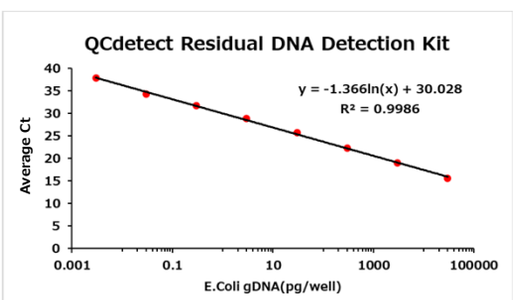
- Limit of detection : ≥ 0.003 pg/test
- Limit of quantitation : ≥ 0.03 pg/test
- Calibration curve with high linearity
- Minimal inter-assay variability and high reproducibility
- Easy operation using the pre-mix buffer
- Less susceptible to impurities in sample
- Contains an internal control

Wako code	Product name		Package Size
290-85301	QCdetect™ Residual DNA Detection Kit for *E. coli*	F	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 0.003 pg to 30,000 pg.

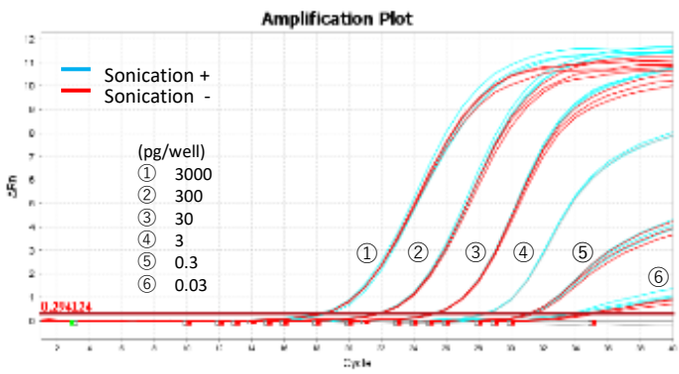


Wako	QCdetect Residual DNA Detection Kit								
pg/well	30000	3000	300	30	3	0.3	0.03	0.003	0.0003
Ct	15.655	19.011	22.335	25.709	28.932	31.941	33.825	37.731	
	15.393	18.976	22.414	25.675	28.875	31.684	34.866	38.012	
	15.713	19.091	22.400	25.696	28.945	31.657	34.507	N.D	N.D
Average Ct	15.587	19.026	22.383	25.693	28.917	31.760	34.399	37.872	
S.E	0.1707	0.0593	0.0418	0.017	0.0371	0.1566	0.5292	0.1989	

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.



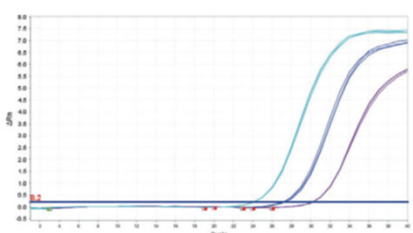
Example of use of this kit for samples containing high-concentration protein – combined with DNA Extractor® Kit –

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.

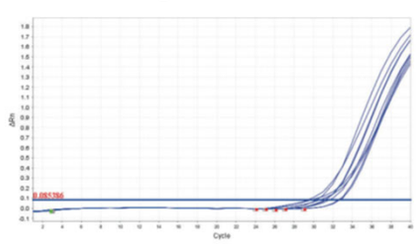
Amount of DNA spiked	0.1 ng/mL	1 ng/mL	10 ng/mL
Spiked DNA amount in the detection reaction (PCR)	1 pg	10 pg	100 pg
Recovery (mean)	96.9%	85.7%	86.9%
SD	0.013	0.67	1.4
CV%	1.3%	7.8%	1.6%

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

Detection of *E. coli*-derived genomic DNA



Detecting Internal Control



Kit contents

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

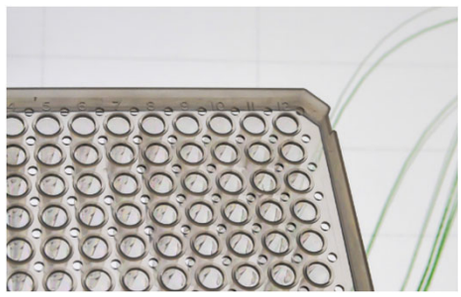
Detection wavelength

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

Note) Internal control is non-natural synthetic DNA.

QCdetect™ Residual DNA Detection Kit for CHO cells

This product is a kit for quantifying genomic DNA from CHO cells by qPCR (probe method).
It can be used for residual DNA testing for antibody pharmaceuticals and their manufacturing processes.



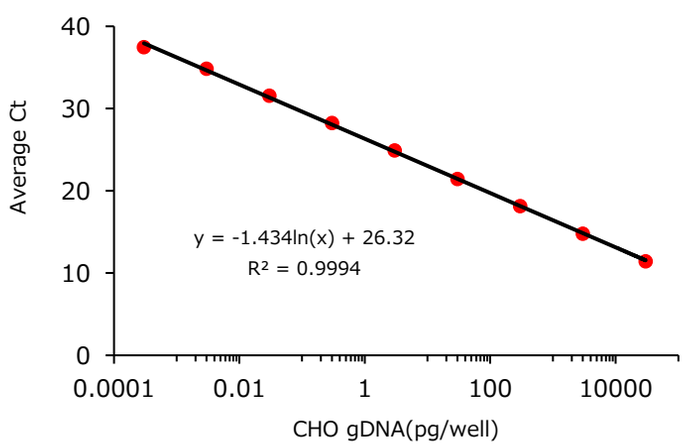
- Limit of detection: ≥ 0.0003 pg/test
- Limit of quantitation: ≥ 0.003 pg/test
- Calibration curve with high linearity
- Minimal inter-assay variability and high reproducibility
- Easy operation using the pre-mix buffer
- Less susceptible to contaminants in samples
- Contains an internal control

Wako code	Product name		Package Size
294-85201	QCdetect™ Residual DNA Detection Kit for CHO cells	F	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.

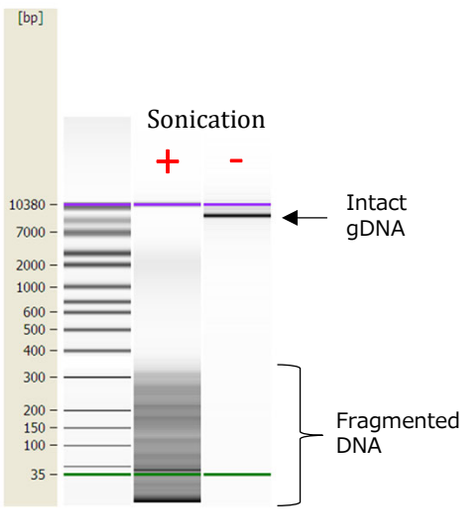


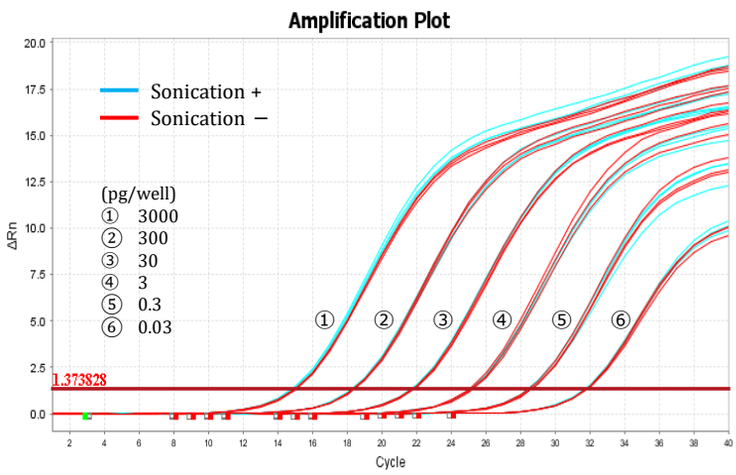
pg/well	0.0003	0.003	0.03	0.3	3	30	300	3000	30000
Ct	37.639	34.822	31.546	28.117	24.809	21.337	18.079	14.726	11.506
	37.107	34.981	31.506	28.324	24.906	21.416	18.084	14.744	11.467
	37.642	34.689	31.595	28.267	24.987	21.533	18.225	14.807	11.269
Average Ct	**37.462**	**34.830**	**31.549**	**28.236**	**24.901**	**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 -

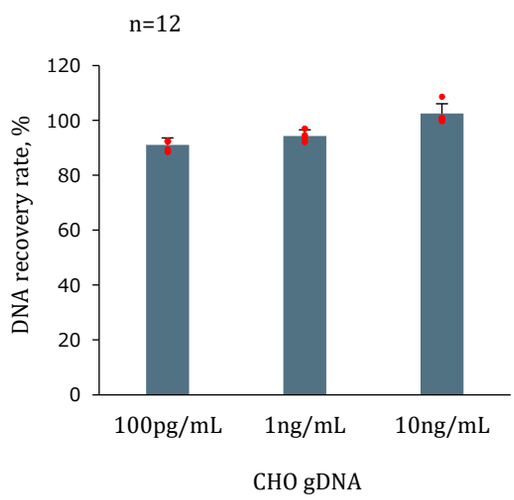
CHO gDNA was spiked to a sample containing high concentration of γ-globulin, and DNA was extracted by DNA Extractor® Kit.
The obtained DNA was quantified with QCdetect™ Residual DNA Detection Kit for CHO cells, and the spike recovery rate was obtained.

▶ Sample composition

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

▶ Concentration of spiked CHO gDNA

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



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

Kit contents

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

Detection wavelength

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

Note) Internal Control is non-natural synthetic DNA.

03

DNA Extractor® Kit

A kit for extracting residual DNA from host cells by sodium iodide method. The extracted DNA can be quantified by qPCR.
This kit can be used for testing and control of the quantity of residual DNA obtained from host cells such as CHO cells, Escherichia coli, and yeast.

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- Trace amounts of DNA (100-1,000 fg) can be recovered in high yield
- 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
- A protocol for high-protein samples is available
- 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."

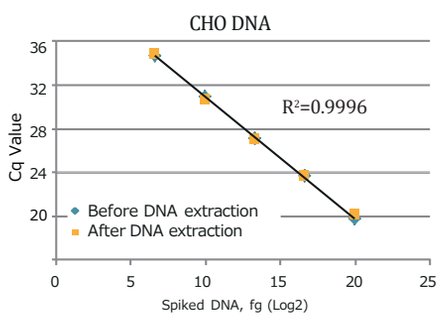
Wako code	Product name		Package Size
295-50201	DNA Extractor® Kit	Ref	50 tests

Example of spike recovery test

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

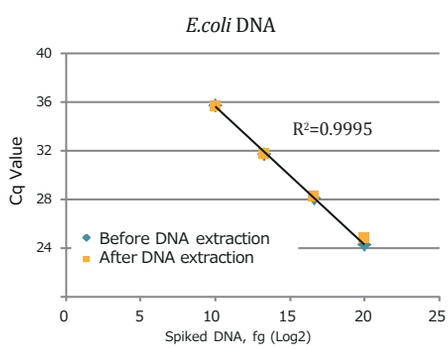
▶ CHO gDNA

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

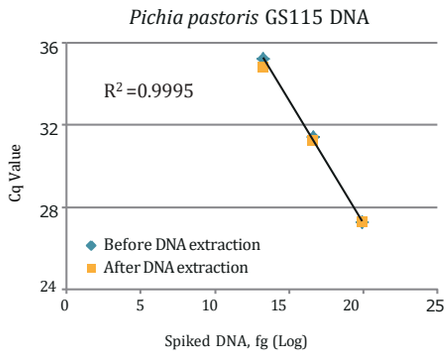
gDNA	*E. coli*	
Spike (fg)	Detection (fg)	Recovery (%)
1,000	939	94
10,000	11,004	110
100,000	100,009	100
1,000,000	969,911	97



▶ *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.

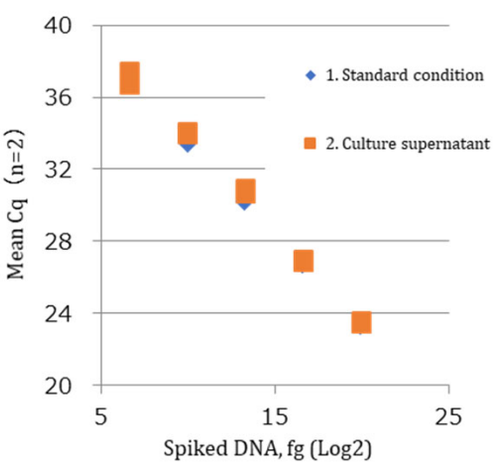


Spike recovery test of CHO gDNA using culture supernatant

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

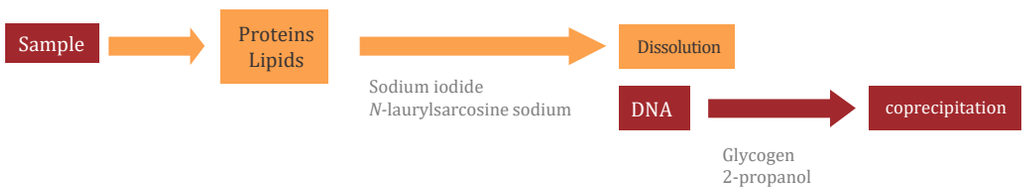
	Culture supernatant	
Spiked amount (fg)	Volume recovered (fg)	Recovery rate (%)
0	ND	
10		
100	93	**93**
1,000	741	**74**
10,000	6,333	**63**
100,000	86,703	**87**
1,000,000	874,502	**87**

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.

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.



Kit contents

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

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