FUJIFILM Wako

产品编号：294-85201（100次用）

QCdetect 残留DNA检测试剂盒说明书
CHO细胞用

<仅供研究使用>

中文版说明书为翻译版本，仅供参考。
内容如有差异，请以英文版为主。

【产品简介】

本产品用于检测和定量疫苗、生物制药等生物来源制剂中残留的DNA。并且，可使用定量PCR法（qPCR）检测和定量中国仓鼠卵巢细胞（CHO cells）来源的基因组DNA（gDNA）。

本试剂盒采用FUJIFILM Wako研发的技术，能高灵敏度检测样品中的微量DNA。为减少制备试剂时出现的人为失误，并提高工作效率和实验精准度，PCR溶液已预调配为1×PCR Master Mix，所以无需额外制备Master Mix。此外，本试剂盒中的qPCR采用了荧光标记探针法（俗称TaqMan® 探针），内含内部对照用（内标）的引物/探针和模板DNA，以确保PCR反应正确进行。

与配套的DNA提取试剂盒（产品编号：292-81101）配合使用，可实现提取和检测连续化，从而检测含有CHO细胞来源生物制剂的蛋白或Buffer成分的溶液中的gDNA。

TaqMan® 是Roche Diagnostics K.K. 的登录商标。

【试剂盒组成】

试剂名称	包装
1×PCR Master Mix	1 mL×2
DNA Dilution Buffer（DDB）	10 mL
CHO Control DNA, 30 ng/µL	40 µL

1×PCR Master Mix还包含用于检测CHO来源gDNA的引物/探针组和内部对照模板DNA和检测用引物/探针组。

【保存温度】-20℃

【允许冻融次数】3次

【使用注意事项】

· 1×PCR Master Mix建议分装为小份保存，避免反复冻融。
· 请遵守实验室规定，进行实验时注意安全。
· 实验时请佩戴好手套和防护眼镜等防护用品。
· 实验前请用乙醇仔细擦拭实验桌具、移液枪枪头等器具。
· 操作过程中，尽量选择污染风险低的场所（如超净工作台，安全柜等）。
· 制备试剂时请置于冰上进行。
· 请勿使用 TE buffer或含有EDTA的buffer。

【除试剂盒以外所需的试剂和器具】

· 实时荧光定量PCR仪
· 漩涡混合器
· 微型离心机
· 无核酸酶灭菌水（例：产品编号：316-90101，日本基因产）
· 微量移液器和无核酸酶移液器枪头
（适用的两种微量移液器：2-20 µL和100-1,000 µL）
· 无核酸酶1.5 mL离心管（推荐使用DNA/RNA低吸附离心管）
· Real-Time PCR板和封板膜，或Real-Time PCR用八联管及管盖
· 消毒用乙醇

【探针的检测波长】

试剂名称	荧光波长
CHO Primer & Probe	FAM
Internal Control Primer & Probe	HEX 或 VIC

【使用方法】

*本说明书的实验方案根据美国药典（USP）的规定制定，若客户有自己的实验方案请结合自己的方案进行实验。

<使用方法1> 用本试剂盒检测已提取的gDNA

使用预提取的gDNA作为模板DNA时的使用方法

1-1. 制备试剂

（1）将试剂盒中的试剂置于冰上溶解。
（2）各试剂用漩涡混合器混合均匀后，用微型离心机离心。

1-2. CHO gDNA阳性对照组制备（制备标准曲线）

（1）准备7支无核酸酶1.5 mL离心管，分别标记为PC1、PC2、PC3、PC4、PC5、PC6、NTC。
NTC（No Template Contol）为阴性对照组
PC（Postive Control）为阳性对照组
（2）往PC1添加990 µL DNA Dilution Buffer(DDB)。
（3）往PC2、PC3、PC4、PC5、PC6、NTC各添加900 µL DDB。
（4）往PC1添加10 µL CHO Control DNA（30 ng/µL）（PC0），混合均匀。
（5）往PC2添加100 µL PC1，混合均匀。
（6）往PC3添加100 µL PC2，混合均匀。
（7）往PC4添加100 µL PC3，混合均匀。
（8）往PC5添加100 µL PC4，混合均匀。
（9）往PC6添加100 µL PC5，混合均匀。

离心管名称	稀释内容	CHO gDNA浓度
PC1	10 µL PC0＋990 µL DDB	3,000 pg/reaction
PC2	100 µL PC1＋900 µL DDB	300 pg/reaction
PC3	100 µL PC2＋900 µL DDB	30 pg/reaction
PC4	100 µL PC3＋900 µL DDB	3 pg/reaction
PC5	100 µL PC4＋900 µL DDB	0.3 pg/reaction
PC6	100 µL PC5＋900 µL DDB	0.03 pg/reaction
NTC	900 µL DDB	0 pg/reaction

1-3. 往Real-Time PCR板添加1×PCR Master Mix

往板上各孔里添加20 µL 1×PCR Master Mix。
（使用Real-Time PCR八联管时，操作相同）。

1-4.添加阳性对照组、阴性对照组和样品

以4种样品（n=3）进行实验为例
（1）如下图所示，往1×PCR Master Mix的各孔里添加于步骤1-2.中制备的阳性对照组、阴性对照组以及样品各10 µL。
（2）用封板膜或管盖封好。

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		PC1				样品1						
C		PC2				样品2						
D		PC3				样品3						
E		PC4				样品4						
F		PC5				NTC						
G		PC6										
H												

1-5. 定量PCR（qPCR）

将孔板置于Real-Time PCR仪后，进行以下程序的设定。
程序设定完成后，开始反应。

Step	反应条件
Hold	95°C，10 min.
Denature	95°C，15 sec.
Annealing & Extension	60°C，1 min.

1-6. 检测

根据PCR仪的使用方法进行操作，确认结果。

1-7. 计算样品DNA

推荐使用Real-Time PCR仪内置的软件进行标准曲线的制备。
如需人手计算，请使用以下方法。

（1）根据标准品（PC1-PC6）的Ct值，制作对数函数图。

（2）根据（1）计算曲线的近似方程。

如果标准曲线不符合以下指标范围，推荐再次实验。

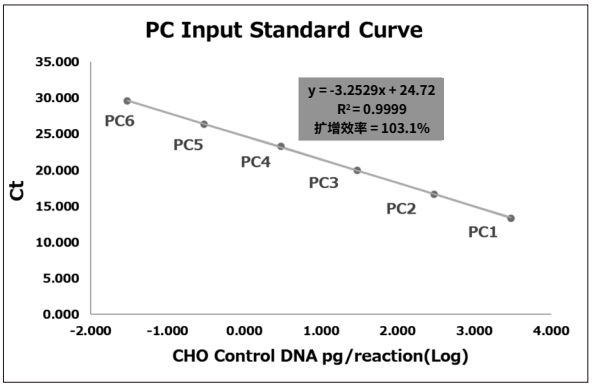
参数	合格值
近似方程的斜率	-3.1~-3.8
R^2值	0.98以上
扩增效率	90~110%
NTC的Ct值	39以上或N.D.

[例]

当实验结果为以下情况时：

Positive Control	PC1 Input	PC2 Input	PC3 Input	PC4 Input	PC5 Input	PC6 Input
pg/reaction	3,000	300	30	3	0.3	0.03
pg/reaction（Log）	3.477	2.477	1.477	0.477	-0.523	-1.523
Ct值	13.335	16.582	19.928	23.278	26.325	29.592
	13.335	16.604	19.960	23.194	26.401	29.519
	13.349	16.675	20.011	23.391	26.427	29.725
平均Ct值	13.346	16.650	19.966	23.288	26.384	29.612

NTC检测为ND。



参数	合格值	结果
标准曲线斜率	-3.1~-3.8	合格
R^2值	0.98以上	合格
扩增效率	90~110%	合格
NTC的Ct值	39以上或N.D.	合格

1-8.结果分析

世界卫生组织[1)]和FDA[2)]对生物制药中的残留DNA设定了以下标准值。

细胞系	DNA容许量
无致瘤性细胞系	10 ng
含有逆转录病毒原病毒序列的细胞系	100 pg

计算样品中残留的DNA含量，确认是否符合上述标准。

此外，推荐在标准曲线所有指标都合格的条件下计算DNA容许量。

<使用方法2>

使用DNA提取试剂盒（产品编号：292-81101）提取gDNA，并配合本试剂盒检测

用DNA提取试剂盒从样品中提取DNA后，使用本试剂盒进行检测时的使用方法。

2-1.制备试剂

（1）将试剂盒中的CHO Control DNA, 30 ng/μL（PC0）置于冰上融解。

（2）漩涡混合器混合均匀后，用微型离心机离心。

2-2.制备CHO gDNA阳性对照组（制备标准曲线）

（1）准备7支1.5 mL无核酸酶离心管，分别标记为PC1、PC2、PC3、PC4、PC5、PC6、NTC。
NTC（No Template Contol）为阴性对照组
PC（Postive Control）为阳性对照组

（2）往PC1添加990 μL DNA Dilution Buffer(DDB)。

（3）往PC2、PC3、PC4、PC5、PC6、NTC各添加900 μL DDB。

（4）往PC1添加10 μL CHO Control DNA（30 ng/μL）（PC0），混合均匀。

（5）往PC2添加100 μL PC1，混合均匀。

（6）往PC3添加100 μL PC2，混合均匀。

（7）往PC4添加100 μL PC3，混合均匀。

（8）往PC5加入100 μL PC4，混合均匀。

（9）往PC6加入100 μL PC5，混合均匀。

离心管名称	稀释内容	CHO gDNA浓度
PC1	10 μL PC0＋990 μL DDB	3,000 pg/reaction
PC2	100 μL PC1＋900 μL DDB	300 pg/reaction
PC3	100 μL PC2＋900 μL DDB	30 pg/reaction
PC4	100 μL PC3＋900 μL DDB	3 pg/reaction
PC5	100 μL PC4＋900 μL DDB	0.3 pg/reaction
PC6	100 μL PC5＋900 μL DDB	0.03 pg/reaction
NTC	900 μL DDB	0 pg/reaction

2-3. 用DNA提取试剂盒（产品编号：292-81101）提取

遵循DNA提取试剂盒的实验方案#2，用500 μL 2-2.制备的DNA溶液进行提取。

2-4. 往Real-Time PCR板添加1×PCR Master Mix

往板上各孔里添加20 μL 1×PCR Master Mix。
（使用Real-Time PCR用八联管时，操作相同。）

2-5. 添加阳性对照组、阴性对照组和样品

以4种样品（n=3）进行实验为例

（1）如下图所示，往1×PCR Master Mix的各孔里添加步骤2-2.和2-3.制备的阳性对照组，阴性对照组以及样品各10 μL。

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		PC1 Input			PC1 Extraction			样品1				
C		PC2 Input			PC2 Extraction			样品2				
D		PC3 Input			PC3 Extraction			样品3				
E		PC4 Input			PC4 Extraction			样品4				
F		PC5 Input			PC5 Extraction			NTC				
G		PC6 Input			PC6 Extraction							
H												

Input：2-2.制备的DNA溶液
Extraction：2-3.提取的DNA溶液
Sample：样品
(2) 用封板膜或管盖封好。

2-6. 定量PCR (qPCR)

将孔板置于Real-Time PCR后，进行以下程序设定。
程序设定完成后，开始反应。

Step	反应条件
Hold	95°C，10 min.
Denature	95°C，15 sec.
Annealing & Extension	60°C，1 min.

2-7. 检测

根据各自PCR仪的使用方法进行操作，确认结果。

2-8. 计算样品DNA

推荐使用Real-Time PCR仪内置的软件进行标准曲线的制作。
如需人手计算，请使用以下方法。
(1) 根据标准品（PC1-PC6）的Ct值制作对数函数图。
(2) 根据(1)计算曲线的近似方程。
如果标准曲线不符合以下指标范围，推荐再次实验。

参数	合格值
标准曲线的斜率	-3.1~-3.8
R^2值	0.98以上
扩增效率	90~110%
NTC的Ct值	39以上或N.D.

(3) 以样品的Ct值为Ct，标准曲线近似方程的斜率为m，截距为B，样品数量计算公式为$10^{(Ct-b/m)}$。
回收率不符合以下指标时，推荐重新实验。

参数	合格值
样品回收率	50~150%
※变异系数	30以下

※标准差除以算术平均数以表示相对变化。
用n=3实验得到数值的标准差×100/平均值

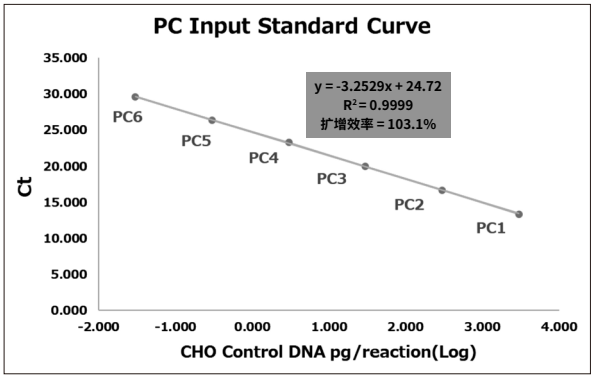
[例]

当实验结果为以下情况时：

Positive Control	PC1 Input	PC2 Input	PC3 Input	PC4 Input	PC5 Input	PC6 Input
pg/reaction	3,000	300	30	3	0.3	0.03
pg/reaction(Log)	3.477	2.477	1.477	0.477	-0.523	-1.523
Ct值	13.335	16.582	19.928	23.278	26.325	29.592
	13.335	16.604	19.960	23.194	26.401	29.519
	13.349	16.675	20.011	23.391	26.427	29.725
平均Ct值	13.346	16.650	19.966	23.288	26.384	29.612

NTC检测结果为ND。

Positive Control	PC1 Extraction	PC2 Extraction	PC3 Extraction	PC4 Extraction	PC5 Extraction	PC6 Extraction
pg/reaction	3,000	300	30	3	0.3	0.03
pg/reaction(Log)	3.477	2.477	1.477	0.477	-0.523	-1.523
Ct值	13.384	16.697	20.030	23.240	26.389	29.622
	13.394	16.574	19.932	23.179	26.304	29.238
	13.327	16.676	20.026	23.206	26.412	29.236
回收量（pg）	3055.058	292.658	27.654	2.851	0.307	0.031
	3033.101	319.379	29.636	2.976	0.326	0.041
	3180.577	296.994	27.744	2.920	0.302	0.041
回收率%	101.835	97.553	92.180	95.032	102.288	103.739
	101.103	106.460	98.787	99.188	108.659	136.098
	106.019	98.998	92.479	97.336	100.659	136.364
平均回收率%	102.986	101.003	94.482	97.186	103.869	125.400
标准差	2.166	3.903	3.046	1.700	3.452	15.317
变异系数	2.043	3.943	3.294	1.747	3.429	11.233



参数	合格值	结果
近似方程的斜率	-3.1~-3.8	合格
R^2值	0.98以上	合格
扩增效率	90~110%	合格
NTC的Ct值	39以上或N.D.	合格
样品回收率	50~150%	合格
变异系数	30%以下	合格

2-9. 结果分析

世界卫生组织[1]和FDA[2]对生物制药中的残留DNA设定了以下标准值。

细胞系	DNA容许量
无致瘤性细胞系	10 ng
含有逆转录病毒原病毒序列的细胞系	100 pg

计算样品中残留的DNA数量，确认是否符合上述标准。
此外，推荐在标准曲线所有指标都合格的条件下计算DNA容许量。

【产品列表】

产品编号	产品名称	包装
294-85201	QCdetect™ Residual DNA Detection Kit for CHO cells	100次用

【相关产品】

产品编号	产品名称	包装
292-81101	DNA Extractor® Kit for Residual DNA, CP Method(Sodium Iodide Method) 残留DNA提取试剂盒（碘化钠法）	50次用

【参考文献】

1. Knezevic, I., Stacey, G., Petricciani, J., and substrates, W. H. O. S. G. o. c. : WHO Study Group on cell substrates for production of biologicals, Geneva, Switzerland, 11-12 June 2007, Biologicals, 36, 203-211（2008）.
2. Points to consider in the manufacture and testing of monoclonal antibody products for human use（1997）. U.S. Food and Drug Administration Center for Biologics Evaluation and Research, J. Immunother., 20, 214-243（1997）.

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FUJIFILM

Wako

Code No. 294-85201 (100 tests)

QCdetect™ Residual DNA Detection Kit for CHO cells

【Introduction】

This product is a kit that detect and quantify DNA remaining in biological products such as vaccines and biopharmacy. In particular, genomic DNA (gDNA) derived from Chinese Hamster Ovary cells (CHO cells) is detected and quantified by the Quantitative PCR method (qPCR).

The unique configuration enables highly sensitive detection of trace amounts of DNA in samples. In addition, the PCR solution is prepared as a 1×PCR Master Mix to suppress human error during preparation and improve work efficiency and test accuracy. Therefore, the customer does not need to prepare the Master Mix for PCR. Furthermore, the qPCR of this kit uses a fluorescein-labeled probe (commonly known as the TaqMan® probe) and contains a primer/probe and template DNA for internal control, so it can be confirmed that PCR reaction is being performed correctly.

By using this kit in combination with the DNA Extractor® Kit [Code No. 295-50201] sold separately, gDNA can be consistently extracted and detected from proteins in biological products using CHO cells or solutions containing buffer components.

TaqMan® is a registered trademark of Roche Diagnostics.

【Kit contents】

Reagent name	Volume
1×PCR Master Mix	1 mL×2 vials
DNA Dilution Buffer (DDB)	10 mL
CHO Control DNA, 30 ng/μL	40 μL

1×PCR Master Mix also contains a primer/probe set for detection of CHO-derived gDNA and a template DNA and a primer/probe set for detection of the internal control.

【Storage】

Store at −20℃.

【Allowable number of freezing and thawing】

3 times

【Before use】

- Divide 1×PCR Master Mix into small portions to reduce the frequency of freezing and thawing.
- Conduct experiments in accordance with the laboratory guidelines and with due attention to safety.
- Wear protective gear including gloves and safety glasses during testing.
- Thoroughly swab the tabletop and pipettes with ethanol before testing.

- Work in a place with the lowest possible risk of contamination (e.g., inside a clean bench, safety cabinet)
- Prepare the reagents on ice.
- Do not use TE buffer or buffer containing EDTA.

【Additional reagents and instruments required】

- Real-time PCR device
- Vortex mixer
- Microtube centrifuge
- Nuclease-free sterile water (e.g., Code No. 316-90101, Nippon Gene)
- Micropipette and nuclease-free pipette tip (Two micropipettes, 2-20 μL and 100-1,000 μL, can be used.)
- Nuclease-free 1.5 mL tube (DNA/RNA low adsorption product is desirable)
- Real-time PCR plate and plate seal, or 8-strip tube for Real-time PCR and tube cap
- Ethanol for disinfection

【Detection wavelength of the probe】

Reagent name	Fluorescence wavelength
CHO Primer & Probe	FAM
Internal Control Primer & Probe	HEX or VIC

【Usage】

*The protocol in this manual has been prepared in accordance with the standards contained in the United States Pharmacopeia (USP). However, please use this kit in accordance with your own protocol if you have one.

[Usage 1 : Detection of gDNA from extracted DNA using this kit]

When the detection kit is used with pre-extracted DNA as template DNA

1-1. Preparation of reagents

(1) Thaw the reagents in the kit on ice.
(2) Mix each reagents well with a vortex mixer, then spin down with a microtube centrifuge.

1-2. Preparation of CHO gDNA-positive control (creation of standard curve)

(1) Prepare 7 nuclease-free 1.5 mL tubes and label them as PC1, PC2, PC3, PC4, PC5, PC6, and NTC, respectively. NTC (No Template Control) stands for negative control.
(2) Add 990 μL of DNA Dilution Buffer (DDB) to PC1.
(3) Add 900 μL of DDB to each of PC2, PC3, PC4, PC5, PC6, and NTC.
(4) Add 10 μL of CHO Control DNA, 30 ng/μL (PC0) to PC1 and mix well.
(5) Add 100 μL of PC1 to PC2 and mix well.
(6) Add 100 μL of PC2 to PC3 and mix well.
(7) Add 100 μL of PC3 to PC4 and mix well.
(8) Add 100 μL of PC4 to PC5 and mix well.
(9) Add 100 μL of PC5 to PC6 and mix well.

Tube name	Diluent	CHO gDNA concentration
PC1	10 μL PC0 + 990 μL DDB	3,000 pg/reaction
PC2	100 μL PC1 + 900 μL DDB	300 pg/reaction
PC3	100 μL PC2 + 900 μL DDB	30 pg/reaction
PC4	100 μL PC3 + 900 μL DDB	3 pg/reaction
PC5	100 μL PC4 + 900 μL DDB	0.3 pg/reaction
PC6	100 μL PC5 + 900 μL DDB	0.03 pg/reaction
NTC	900 μL DDB	0 pg/reaction

1-3. Addition of 1×PCR Master Mix to Real-Time PCR plate

Add 20 μL of 1×PCR Master Mix to each plate well.
(Do the same when using 8-strip tube.)

1-4. Addition of positive control, negative control, and sample

An example of testing four types of samples (n=3) is shown below.

(1) Add 10 μL of the positive and negative control prepared in 1-2. and the sample to each well containing 1×PCR Master Mix as shown below.
(2) Cover with a plate seal or cap.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		PC1				Sample 1						
C		PC2				Sample 2						
D		PC3				Sample 3						
E		PC4				Sample 4						
F		PC5				NTC						
G		PC6										
H												

1-5. Quantitative PCR (qPCR)

Place the plate in the Real-time PCR device and set up the program shown below.
After setting up the program, start the reaction.

Step	Reaction conditions
Hold	95℃, 10 min.
Denature	95℃, 15 sec.
Annealing & Extension	60℃, 1 min.

1-6. Detection

Check the results in accordance with the device protocol.

1-7. Calculation of DNA content in the sample

Creating a standard curve with the software of the Real-time PCR device is recommended.
When calculating by hand, use the following method:

(1) Create a logarithmic graph from the Ct value of the standard (PC1-PC6).
(2) Calculate the approximate equation of the curve from (1). Repeat testing is recommended if there is a deviation in any of the following standard curve parameters :

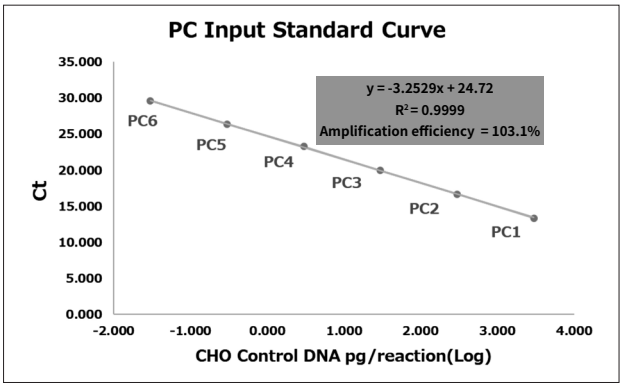
Condition	Proper value
Slope of approximate equation	−3.1 to −3.8
R^2 value	≥ 0.98
Amplification efficiency	90% to 110%
Ct value of NTC	≥ 39 or N.D.

[Example]

When the test results are as follows :

Positive Control	PC1 Input	PC2 Input	PC3 Input	PC4 Input	PC5 Input	PC6 Input
pg/reaction	3,000	300	30	3	0.3	0.03
pg/reaction (Log)	3.477	2.477	1.477	0.477	−0.523	−1.523
Individual Ct value	13.335	16.582	19.928	23.278	26.325	29.592
	13.355	16.604	19.960	23.194	26.401	29.519
	13.349	16.675	20.011	23.391	26.427	29.725
Mean Ct value	13.346	16.650	19.966	23.288	26.384	29.612

NTC should be detected as ND.



Condition	Proper value	Proper result
Slope of standard curve	−3.1 to −3.8	Acceptable
R^2 value	≥ 0.98	Acceptable
Amplification efficiency	90% to 110%	Acceptable
Ct value of NTC	≥ 39 or N.D.	Acceptable

1-8. Interpretation of results

The WHO[1] and the FDA[2] have established the following criteria for residual DNA during biopharmaceutical manufacturing :

Cell type	Acceptable amount of DNA
Non-tumorigenic cell lines	10 ng
Cell lines with retrovirus proviral sequence	100 pg

Calculate the amount of DNA remaining in the sample to confirm that it does not exceed the acceptable amount.
In addition, it is recommended that the acceptable amount of DNA be calculated under the condition where all results of the standard curve are acceptable.

[Usage 2 : When this kit is used with gDNA extracted with DNA Extractor® Kit]

When gDNA is detected with this kit after DNA is extracted with DNA Extractor® Kit [Code No. 295-50201].

2-1. Preparation of reagents

(1) Thaw CHO Control DNA, 30 ng/μL (PC0) in the kit on ice.
(2) Mix it well with a vortex mixer, then spin down with a microtube centrifuge.

2-2. Preparation of CHO gDNA-positive control (creation of standard curve)

(1) Prepare 7 nuclease-free 1.5 mL tubes and label them as PC1, PC2, PC3, PC4, PC5, PC6, and NTC, respectively. NTC (No Template Control) stands for negative control.
(2) Add 990 μL of DNA Dilution Buffer (DDB) to PC1.
(3) Add 900 μL of DDB to each of PC2, PC3, PC4, PC5, PC6, and NTC.
(4) Add 10 μL of CHO Control DNA, 30 ng/μL (PC0) to PC1 and mix well.
(5) Add 100 μL of PC1 to PC2 and mix well.
(6) Add 100 μL of PC2 to PC3 and mix well.
(7) Add 100 μL of PC3 to PC4 and mix well.
(8) Add 100 μL of PC4 to PC5 and mix well.
(9) Add 100 μL of PC5 to PC6 and mix well.

Tube name	Diluent	CHO gDNA concentration
PC1	10 μL PC0 + 990 μL DDB	3,000 pg/reaction
PC2	100 μL PC1 + 900 μL DDB	300 pg/reaction
PC3	100 μL PC2 + 900 μL DDB	30 pg/reaction
PC4	100 μL PC3 + 900 μL DDB	3 pg/reaction
PC5	100 μL PC4 + 900 μL DDB	0.3 pg/reaction
PC6	100 μL PC5 + 900 μL DDB	0.03 pg/reaction
NTC	900 μL DDB	0 pg/reaction

2-3. Extraction with DNA Extractor® Kit

Extract DNA from 500 μL of each of the DNA solutions prepared as described in 2-2. in accordance with protocol #2 of the DNA Extractor® Kit.

2-4. Addition of 1×PCR Master Mix to Real-Time PCR plate

Add 20 μL of 1×PCR Master Mix to each plate well.
(Do the same when using 8-strip tube.)

2-5. Addition of positive control, negative control, and sample

An example of testing four types of samples (n=3) is shown below.

(1) Add 10 μL of the positive and negative control prepared as described in 2-2. and 2-3. and the sample to each well containing 1×PCR Master Mix as shown below.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		PC1 Input			PC1 Extraction			Sample 1				
C		PC2 Input			PC2 Extraction			Sample 2				
D		PC3 Input			PC3 Extraction			Sample 3				
E		PC4 Input			PC4 Extraction			Sample 4				
F		PC5 Input			PC5 Extraction			NTC				
G		PC6 Input			PC6 Extraction							
H												

Input : DNA solution prepared as described in 2-2.
Extraction : DNA solution purified as described in 2-3.
Sample : Test

(2) Cover with a plate seal or cap.

2-6. Quantitative PCR (qPCR) reaction

Place the plate in the Real-time PCR device and set up the program shown below.
After setting up the program, start the reaction.

Step	Reaction conditions
Hold	95℃, 10 min.
Denature	95℃, 15 sec.
Annealing & Extension	60℃, 1 min.

2-7. Detection

Check the results in accordance with the device protocol.

2-8. Calculation of DNA content in the sample

Creating a standard curve with the software of Real-time PCR device is recommended.
When calculating by hand, use the following method :

(1) Create a logarithmic graph from the Ct value of the standard (PC1-PC6).
(2) Calculate the approximate equation of the curve from (1).
Repeat testing is recommended if there is a deviation in any of the following standard curve parameters :

Condition	Proper value
Slope of standard curve	−3.1 to −3.8
R^2 value	≥ 0.98
Amplification efficiency	90% to 110%
Ct value of NTC	≥ 39 or N.D.

(3) The amount of DNA in the sample is calculated by the formula $10^{(Ct-b/m)}$, where Ct is the Ct value of the sample, and m and b are the slope and intercept of the approximate equation of the standard curve, respectively.
Repeat testing is recommended if there is a deviation in any of the following recovery rate parameters :

Condition	Proper value
Sample recovery rate	50% to 150%
*Coefficient of variation	< 30%

*The coefficient of variation is the standard deviation divided by the arithmetic mean and indicates relative variability.

Standard deviation × 100/mean when tested with n=3

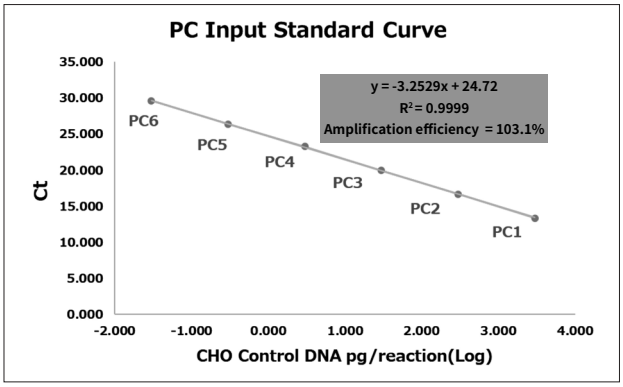
【Example】

When the test results are as follows :

Positive Control	PC1 Input	PC2 Input	PC3 Input	PC4 Input	PC5 Input	PC6 Input
pg/reaction	3,000	300	30	3	0.3	0.03
pg/reaction (Log)	3.477	2.477	1.477	0.477	−0.523	−1.523
Individual Ct value	13.335	16.582	19.928	23.278	26.325	29.592
	13.355	16.604	19.960	23.194	26.401	29.519
	13.349	16.675	20.011	23.391	26.427	29.725
Mean Ct value	13.346	16.650	19.966	23.288	26.384	29.612

NTC should be detected as ND.

Positive Control	PC1 Extraction	PC2 Extraction	PC3 Extraction	PC4 Extraction	PC5 Extraction	PC6 Extraction
pg/reaction	3,000	300	30	3	0.3	0.03
pg/reaction (Log)	3.477	2.477	1.477	0.477	-0.523	-1.523
Individual Ct value	13.384	16.697	20.030	23.240	26.389	29.622
	13.394	16.574	19.932	23.179	26.304	29.238
	13.327	16.676	20.026	23.206	26.412	29.236
Amount recovered individually (pg)	3,055.058	292.658	27.654	2.851	0.307	0.031
	3,033.101	319.379	29.636	2.976	0.326	0.041
	3,180.577	296.994	27.744	2.920	0.302	0.041
Individual recovery rate (%)	101.835	97.553	92.180	95.032	102.288	103.739
	101.103	106.460	98.787	99.188	108.659	136.098
	106.019	98.998	92.479	97.336	100.659	136.364
Mean recovery rate (%)	102.986	101.003	94.482	97.186	103.869	125.400
Standard deviation	2.166	3.903	3.046	1.700	3.452	15.317
Coefficient of variation (%)	2.043	3.943	3.294	1.747	3.429	11.233



Condition	Proper value	Proper result
Slope of approximate equation	−3.1 to −3.8	Acceptable
R^2 value	≥ 0.98	Acceptable
Amplification efficiency	90% to 110%	Acceptable
Ct value of NTC	≥ 39 or N.D.	Acceptable
Sample recovery rate	50% to 150%	Acceptable
Coefficient of variation	< 30%	Acceptable

2-9. Interpretation of results

The WHO[1] and the FDA[2] have established the following criteria for residual DNA during biopharmaceutical manufacturing.

Cell type	Acceptable amount of DNA
Non-tumorigenic cell lines	10 ng
Cell lines with retrovirus proviral sequence	100 pg

Calculate the amount of DNA remaining in the sample to confirm that it does not exceed the acceptable amount.
In addition, it is recommended that the acceptable amount of DNA be calculated under the condition where all results of the standard curve and sample recovery are acceptable.

【Related Product】

Code No.	Description	Size
295-50201	DNA Extractor® Kit	50 tests

【References】

1. Knezevic, I., Stacey, G., Petricciani, J., and substrates, W. H. O. S. G. o. c. : WHO Study Group on cell substrates for production of biologicals, Geneva, Switzerland, 11-12 June 2007, *Biologicals*, **36**, 203-211 (2008).
2. Points to consider in the manufacture and testing of monoclonal antibody products for human use (1997). U.S. Food and Drug Administration Center for Biologics Evaluation and Research, *J. Immunother.*, **20**, 214-243 (1997).

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