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E. coli Host Cell DNA Residue Detection Kit

Cat No.:ECD-HCD

Size: 50T / 100T

Application: Quantitative analysis of DNA residue in recombinant protein expressed products, purified intermediate and finished products from host cell.

For Research, Development and Manufacturing Use Only!

Not for Therapeutic Or Diagnostic Applications!

1. Introduction

This kit adopts Taqman probe fluorescence qPCR method. The kit has the advantages of high specificity and sensitivity by using specific primers & probes, LOQ can reach 30fg/ μ L level. This kit can be used in combination with our Magnetic Residual DNA Sample Preparation Kit (Cat#CG-HCD100T).

The preparation process of DNA Control is completely consistent with National Standard, therefore it has high purity and no protein and ion interference. DNA Control has been calibrated by National Standard to ensure the accuracy of the sample quantitative detection.

The kit provides DNA Dilution Buffer, which enables good replicate parallelism in a single experiment and good reproducibility between multiple experiments.

2. Kit Components

DNA Amplification			
Components NO.	**Components Name**	**Size**	
		50T	100T
B1	2XqPCR Mix	0.625mL	1.25mL
B2	Primer&Probe Mix	100μL	200μL
B3	DNA Dilution Buffer	2×1.5mL	4×1.5mL
B4	DNA Control (30ng/μL)	25μL	50μL
B5	RNase-Free H2O	0.5mL	1mL
B6	50X ROX Reference Dye(Optional)	0.15ml	0.3ml

**The usage of ROX reference dye is optional and depends on the type of instrument being used. Please refer to the details in PART 6*

3. Equipment Required But Not Provided

1.	Pipette: 5ul-1000ul	**5.**	Mini Centrifuge
2.	1.5/2ml RNase-/DNase-free Centrifuge Tube	**6.**	DNase/RNase-free 8-Tube Strip
3.	200uL RNase-/DNase-free PCR Tube	**7.**	Biological Safety Cabinet Class 2
4.	Vortex Mixer	**8.**	Fluorescence qPCR Detection System

4. Shipping and Storage

1) All components are shipped on dry ice.
2) The kit should be stored at -20℃ and it is recommended to be used within one year. B2 should be stored protected from light.
3) B2/B3/B4 can be stored at -20℃ for 2 years, while B1/B5/B6 can be stored at -20℃ for 1 year. B1/B5/B6 can also be purchased together as a separate set.

5. Preparation Before Experiment

1) Please read the manual thoroughly before use. All the components should be completely thawed, centrifuged at low speed, and vortexed well before use.

2) Avoiding the formation of bubbles, gently invert B1 (2XqPCR Mix) and B6 (50X ROX Reference Dye) after thawing them separately from -20° C storage. Use the solution only after complete homogenization. **Note**: If component B1and B6 will be frequently used for a period of time, they can be stored at 2-8° C for up to 3 months. Avoid repeated freeze-thaw cycles as much as possible. If not used after thawing, thoroughly mix before refreezing.

3) Do not pipette up and down repeatedly when mixing B2 (Primer & Probe Mix) and B4 (DNA Control) before use. Instead, a technique similar to cleaning the walls of the tube can be used to ensure the standard is evenly mixed. Note: To reduce the number of freeze-thaw cycles and avoid contamination, it is recommended to aliquot and store B4 (DNA Control) at -20° C upon first use.

4) The thawed but unused B3 (DNA Dilution Buffer) can be stored at 2-8° C for up to 7 days. If not used for an extended period, it should be stored at -20° C to maintain stability.

5) For your safety and health, please wear lab coat and disposable gloves when conducting the experiment. UV irradiation for 30 minutes before and after the experiment is recommended to eliminate potential DNA contamination in the environment.

6) Due to the high sensitivity of fluorescence quantitative PCR experiments, it is very important to maintain a clean working environment. Before starting the experiment, it is recommended to thoroughly clean the pipette and the surrounding work area, and remove any unnecessary items during the experiment.

6. ASSAY PROCEDURE

1) Dilution of DNA Control and Preparation of Standard Curves

   (1) Thaw B4 (DNA Control) and B3 (DNA Dilution Buffer) on ice, mix gently, and centrifuge at low speed for 10 seconds.

   (2) Take 6 clean 200ul PCR tubes, and label them as S0, S1, S2, S3, S4, and S5. Add 45μL of DNA Dilution Buffer to each tube.

   (3) Add 5μL of DNA Control (30 ng/μL) to S0 to dilute to 3000pg/μL. Centrifuge for 10 seconds, vortex for 5 seconds, then centrifuge again for 10 seconds. This concentration can be aliquoted and stored at -20℃ for short-term use (up to 3 months). Avoid repeated freeze-thaw cycles as much as possible.

   (4) Dilution procedure in S1, S2, S3, S4, and S5 tubes follows the same procedure as S0:

Tube	Dilution Process	Final Standard Concentration
S0	5μL DNA Control (30ng/μL) + 45 μL DNA Dilution Buffer	3000pg/μL
S1	5μL S0 + 45μL DNA Dilution Buffer	300pg/μL
S2	5μL S1 + 45μL DNA Dilution Buffer	30pg/μL
S3	5μL S2 + 45μL DNA Dilution Buffer	3pg/μL
S4	5μL S3 + 45μL DNA Dilution Buffer	300fg/μL
S5	5μL S4 + 45μL DNA Dilution Buffer	30fg/μL

2) PCR Reaction System

Components	Volume(μL)
2XqPCR Mix	12.5
Primer&Probe Mix	2
DNA template（control or sample）	5
Add water	5.5
Total Volume	25

NOTES:

- Calculate the total volume of Mix solution required for this reaction based on the number of reaction wells: Mix solution = (number of reaction wells+4) * (12.5+2+5.5) μ L (including the volume lost in the 4 wells). It is recommended to perform the operation on ice.
- Standards and samples are recommended to be tested in triplicate. The detection range of the standard curve mentioned above is suitable for most experiments and can be adjusted as needed.
- Maintain consistent experimental procedures. Seal the tube after sample addition, then centrifuge at low speed for 10 seconds to collect the liquid to the bottom. Vortex for at least 5 seconds to ensure complete mixing. Centrifuge again at low speed for 10 seconds. Remove the bubbles that may occurs.
- To ensure the accuracy of experimental results, we recommend diluting the protein concentration to 1-10mg/ml using 1X PBS for spike recovery experiments, which will ensure the recovery rate falls within the range of 50% to 150%.
- Matching concentration for ROX Reference Dye of several instruments are listed in the table below.:

Instrument	Concentration
ABI PRISM 7000/7300/7700/7900HT/Step	2.5X（e.g. 1.25μL ROX/25μL system）
ABI 7500/7500Fast Stratagene Mx3000P/Mx3005P/Mx4000	0.5X（e.g. 0.25μL ROX/25μL system）
Roche/Bio-Rad/Eppendorf	No need to add

(1) Data Settings for PCR Instrument (Two-Step Method)

Phase	Temp.(℃)	Time	Content	Fluorescence Signal Collection	Cycles
Pre-denaturation	95℃	15mi	Pre-denaturation	NO	1
PCR Reaction	95℃	3sec	Denaturation *	NO	40
	60℃	30sec	Annealing/Extension *	YES	

NOTES:

- The pre-denaturation condition for PCR reaction must be set at 95 ℃ for 15 minutes.
- Select Reporter as "FAM", Quencher as "TAMRA".
- Follow the instrument user manual for time setting on different models. Set the time as 1 sec. for ABI 7900HT/7900HT Fast/ViiA 7/StepOnePlus.

- Annealing/Extension *: Follow the instrument user manual for time setting on different models. The time settings for several common instruments are listed in the table below:

Instrument	Time Setting
ABI 7500 Fast/7900HT/7900HT Fast/ViiA 7/StepOne/StepOnePlus	30 sec
Roche LightCycle/LightCycle 480	20 sec
ABI 7000/7300	31 sec
ABI 7500	32 sec

7. Criteria for Results

1) Standard Curve: $R^2 > 0.99$; Amplification Efficiency: 90% ≤ Eff% ≤ 110%; Slope: -3.8~-3.1.
2) The recovery rate of spiked samples=(measured value of spiked samples - measured value of samples)/theoretical value of spiked samples * 100%, with a range of 50% -150%.
3) No Template Control (NTC): In the reaction system, replacing the target template with DNA Dilution Buffer while keeping other components unchanged, and the Ct value obtained should be 'Undetermined' or Ct value≥35.

大肠杆菌 宿主细胞 DNA 残留检测试剂盒-操作说明书

应用：定量检测重组蛋白表达、中间产物纯化及最终成品中残留 DNA 的含量

本试剂盒仅供科研和生产使用，不得用于临床及诊断！

规格：

50T/100T

一、产品介绍

本试剂盒采用 Taqman 探针荧光定量 PCR 法，设计专属探针和关键引物，特异性好，灵敏度高，其最低检测限可以达到 30fg/μL 水平。本试剂盒可与本公司的磁珠法残留 DNA 样本前处理试剂盒(Cat#CG-HCD100T)配套使用。

DNA 参考品制备过程与国家标准品制备完全一致，因此纯度高，无蛋白及离子干扰，经国家标准品浓度校正，保证了待测样品含量检测的准确度。

试剂盒提供 DNA 稀释专用稀释液，单次实验复孔平行性及多次实验间数据重现性好。

二、试剂盒组成

DNA 扩增			
组分编号	组分名称	规格	
		50T	100T
B1	2X qPCR Mix	0.625mL	1.25mL
B2	Primer & Probe Mix	100 μL	200 μL
B3	DNA Dilution Buffer	2×1.5mL	4×1.5mL
B4	DNA Control (30ng/ μL)	25 μL	50 μL
B5	RNase-Free H2O	0.5mL	1mL
B6	50X ROX Reference Dye（可选）	0.15ml	0.3ml

* ROX 参比染料为可选，是否使用取决于使用的仪器。具体可参照 PART 6 中的说明。

三、实验需要但未提供的耗材及设备

1. 移液器：5ul-1000ul
2. 1.5/2ml 无 DNA 酶/RNA 酶离心管
3. 200μL 无 DNA 酶/RNA 酶 PCR 管
4. 涡旋仪
5. 迷你离心机
6. 无 DNA 酶/RNA 酶 8 联管
7. 生物安全柜
8. 荧光定量 qPCR 仪

四、运输和保存方法

1) 所有组分均干冰运输.
2) 试剂盒需-20℃保存，建议一年内使用完。其中组分 B2 需避光保存。

3) B2/B3/B4 组分在-20℃可以保存两年，B1/B5/B6 组分在-20℃可以保存一年。此外 B1/B5/B6 三个组分也可以一起另外购买。

五、实验前准备

1) 使用本试剂前请仔细阅读说明书，所有成分使用前应完全化冻，低速离心，震荡混匀。
2) 从-20℃冰箱取出，将组分 B1(2X qPCR Mix)和 B6（50X ROX Reference Dye）分别融解，轻轻颠倒（尽量避免产生泡沫），待溶液完全均一后再行使用。注：如 B1(2X qPCR Mix)和 B6（50X ROX Reference Dye）需一段时间内经常取用，可在 2-8 度条件下储存 3 个月。尽量避免反复多次冻融；如解冻后没有使用，须彻底混匀后重新冷冻。
3) B2 (Primer & Probe Mix) and B4 (DNA Control)在使用前混匀时切勿反复吹打，可采用类似清洗管壁方式。注：为减少反复冻融次数和避免污染，建议初次使用时将 B4 (DNA Control)分装储存于-20℃。
4) 已融化未使用的 B3 (DNA Dilution Buffer)可保存于 2-8℃ 7 天，若长时间不用，请放置于-20℃。
5) 为了您的安全和健康，请穿实验服并戴一次性手套操作，实验开始前后紫外照射 30min 以消除环境中潜在的 DNA 污染源。
6) 由于荧光定量 PCR 实验有极强的灵敏度，保持工作环境的洁净非常重要，实验开始前建议完全清洁移液器及周边工作环境，移除清理实验过程中用不到的任何物品。

六、操作过程

（一）DNA 定量参考品的稀释和标准曲线的制备

1) 将试剂盒中的 DNA Control 和 DNA Dilution Buffer 置于冰上完全融化，轻微振荡混匀，低速离心 10 sec。

2) 取 6 支洁净的 200ul PCR 管，分别标记为 S0，S1，S2，S3，S4，S5，每管各加入 45 μ L DNA 稀释液。

3) 在标记为 S0 的 PCR 管中加入 5μL DNA Control (30 ng/μL)，即 3000pg/μL，快速离心 10 sec，振荡 5sec，再快速离心 10 sec，该浓度可分装置于-20℃短期保存（不超过 3 个月），使用时避免反复冻融。

4) S1，S2，S3，S4，S5 管稀释操作同 S0，稀释方法如下：

	稀释过程	终浓度
S0	5μL DNA Control (30ng/μL) + 45 μL DNA Dilution Buffer	3000pg/μL
S1	5μL S0 + 45μL DNA Dilution Buffer	300pg/μL

S2	5μL S1 + 45μL DNA Dilution Buffer	30pg/μL
S3	5μL S2 + 45μL DNA Dilution Buffer	3pg/μL
S4	5μL S3 + 45μL DNA Dilution Buffer	300fg/μL
S5	5μL S4 + 45μL DNA Dilution Buffer	30fg/μL

（二）反应体系

组分	体积（μL）
2X qPCR Mix	12.5
Primer&Probe Mix	2
DNA template（control or sample）	5
补加水	5.5
总体积	25

【注】：

- 根据反应孔数计算本次所需 Mix 混合液总量：Mix 混合液=（反应孔数+4）*（12.5+2+5.5）μL（含有 4 孔的损失量），建议冰上操作。
- 标准品及待测样本每个浓度做 3 个复孔。上述标准曲线的线性范围适用大多数实验，可根据实际需要适当调整等。
- 实验操作应保持一致。加样完成密封好管子后，请低速离心 10 sec 将管壁的液体离心收集至管底，再震荡混匀 5 sec 以上，完全混匀反应液，再低速离心 10 sec，如有气泡，需将气泡排尽。
- 为确保实验结果准确性，建议用 1X PBS 将溶液中蛋白浓度稀释至 1-10mg/ml 进行加标回收率实验，确保回收率在 50%~150%之间。
- 几种常见仪器的匹配 ROX Reference Dye 浓度见下表：

仪器	终浓度
ABI PRISM 7000/7300/7700/7900HT/Step One 等	2.5X（例如：1.25μL ROX/25μL 体系）
ABD 7500/7500Fast Stratagene Mx3000P/Mx3005P/Mx4000 等	0.5X（例如：0.25μL ROX/25μL 体系）
Roche/Bio-Rad/Eppendorf 仪器等	不用添加

（三）扩增程序设置（2 步法）

阶段	温度(℃)	时间	内容	荧光信号采集	循环数
预变性	95℃	15 min	预变性	否	1
PCR 反应	95℃	3 sec	变性*	否	40
	60℃	30 sec	退火/延伸*	是	

【注】：

- PCR 反应的预变性条件必须设定为 95℃，15min。
- 选择报告荧光基团为“FAM”，猝灭荧光基团为“TAMRA”。
- 变性*：请按照仪器使用说明书对不同型号的仪器进行时间。使用 ABI 7900HT/7900HT Fast/ViiA 7/StepOnePlus 时可设定为 1sec。
- 退火/延伸*：请按照仪器使用说明书对不同型号的仪器进行时间。几种常见仪器的时间设定见下表：

使用 ABI 7500 Fast/7900HT/7900HT Fast/ViiA 7/StepOne/StepOnePlus 时请设定在 30sec
使用 Roche LightCycler/LightCycler 480 时请设定在 20sec
使用 ABI 7000/7300 时请设定在 31sec
使用 ABI 7500 时请设定在 32sec

七、判定标准

1) 标准曲线：R^2>0.99；扩增效率(Eff%)：90%≤Eff%≤110%；斜率（Slope）：-3.8~-3.1。
2) 加标回收率%＝(加标样品测定值－样品测定值)/加标理论值*100%，范围 50%-150%。
3) 无模板对照（NTC）：即反应体系中用 DNA 稀释液代替待检测模板，其余成分不变，其检测结果 Ct 值应为 Undetermined 或 Ct 值≥35。