产品使用说明书

产品名称

全能核酸酶残留检测试剂盒 （Intelli Nuclease ELISA Kit）

规格

96T/盒

预期用途

本试剂盒用于定量检测样本中全能核酸酶的含量

检测原理

本试剂盒采用双抗体夹心法原理，酶标板微孔中包被抗全能核酸酶抗体，加入样本后孵育洗涤，再加入辣根过氧化物酶（HRP）标记抗体孵育，形成抗体-抗原-酶标抗体复合物，经过彻底洗涤后加底物 TMB 显色，TMB 在过氧化物酶的催化下转化成蓝色，经酸的终止作用转化成最终的黄色。颜色的深浅和样品中全能核酸酶含量呈正相关。用酶标仪在450nm波长下测定吸光度（OD 值），根据标准曲线计算样品中全能核酸酶浓度。

试剂盒组成

名称	规格	数量
反应板	8×12 孔	1
酶结合物（100×）	0.15mL	1
稀释液	30mL	1
显色剂	6mL	2
终止液	6mL	1
浓缩洗涤液（10×）	50mL	1
浓缩标准品	0.5mL	1
封板膜	-	4
说明书	-	1

储存条件及有效期

1、2~8℃储存12个月

2、开封后需在6周内用完

所需仪器

1、酶标仪（含450nm波长滤光片，建议含有双波长检测模式，主副波长分别为 450nm和650nm）

2、微孔板振荡器（400-600rpm）

检测流程图

试剂准备

1、将试剂盒平衡至室温（18~25°C）。

2、10×浓缩洗涤液用纯化水按 1:9 体积比稀释成洗涤工作液。

3、将 100×酶结合物用稀释液作 100 倍稀释后使用。

4、浓缩标准品（1000ng/mL）用稀释液稀释至20ng/mL、10ng/mL、5ng/mL、2.5ng/mL、1.25ng/mL 、0.63ng/mL 、0.31ng/mL 、0ng/mL，

稀释方法建议如下：

终浓度		稀释方法	
编号	（ng/mL）	稀释液	工作标准品加量
A	20	0.98mL	20μL 浓缩标准品
B	10	0.5mL	0.5mL A 溶液
C	5	0.5mL	0.5mL B 溶液
D	2.5	0.5mL	0.5mL C 溶液
E	1.25	0.5mL	0.5mL D 溶液
F	0.63	0.5mL	0.5mL E 溶液
G	0.31	0.5mL	0.5mL F 溶液
H	0	0.5mL	/

检测步骤

1、从室温平衡后的铝箔袋中取出所需板条，剩余板条用自封袋密封放回 2~8°C。

2、设置标准品孔和样本孔，建议所有标准品和待测样本进行双复孔测定，标准品孔各加入不同浓度的标准品 100μL，样本孔中加入待测样本100μL。

3、用封板膜封住反应孔，室温下振板（500rpm）反应60min。

4、弃去液体，吸水纸上拍干，每孔加满洗涤液（300μL），静置15~30s，甩去洗涤液，吸水纸上拍干，如此重复洗板5次。

5、标准品孔和样本孔中每孔加入工作浓度的酶结合物 100μL，用封板膜封住反应孔，室温下振板（500rpm）反应30min。

6、弃去液体，吸水纸上拍干，每孔加满洗涤液（300μL），静置15~30s，甩去洗涤液，吸水纸上拍干，如此重复洗板5次。

7、每孔加入显色剂 100uL，用封板膜封住反应孔，室温静置避光反应 30min。

8、每孔加入终止液 50μL，15min内进行检测，设定酶标仪波长于450nm处（建议用双波长450nm/650nm）。

检测结果的解释

1、取标准品、空白对照、样本的平均光吸收值，减去空白对照的平均光吸收值，得到标准品、样品的光吸收校准值。以标准品浓度为横 坐标，校准后的标准品光吸收值为纵坐标绘制标准曲线。（双波长检测模式下的光吸收值为450nm减去650nm）。

2、多种绘图和统计学软件可以用于辅助绘制 标准曲线并进行未知样本浓度的计算。三次曲线拟合法往往曲线拟合效果较好，但其它方法如 Logistic（4P）也可能获得较好拟合结果，需要根据具体实验数据进行分析。

检测性能

1、检出限：0.06 ng/mL

2、检测低限：0.2 ng/mL

3、线性范围：0.2~20 ng/mL

4、精密度：批内变异系数≤10%，批间变异系数≤15%

5、回收率：80%~120%

6、特异性：本试剂盒搭配多宁Intelli核酸酶使用，也可用于其他厂家全能核酸酶的检测，但应该使用相应的产品建立标准曲线

注意事项

1、显色温度和时间对实验结果至关重要，应准确把握

2、洗涤过程中应使洗涤液浸泡反应板15~30s 后再甩干，以充分洗涤非特异性吸附的成分

3、所有试剂使用前应充分摇匀，加样时应将所加样物加入酶标板孔中底部，避免加在孔壁上部，加样时注意不可溅出，不可产生气泡

4、若发现浓缩洗涤液中有结晶，可在37°C水浴锅中孵育，待结晶完全溶解后再混匀稀释至工作浓度

5、样本中应避免引入叠氮钠（NaN3），叠氮钠会破坏辣根过氧化物酶活性，使检测值偏低

技术支持

有任何技术问题或技术支持，请联系：marketing@duoningbio.com

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Protocol Booklet for Intelli Nuclease ELISA Kit

Product Name

Intelli Nuclease ELISA Kit

Specification

96T/kit

Intended Purpose

The kit is for quantitative detection of the content of omnipotent nuclease in samples.

Principle of Test

The kit is designed on the basis of double antibody sandwich principle. The wells of its ELISA plate are coated with anti-omnipotent nuclease antibody. Upon adding the samples for inoculation and washing, adding horseradish peroxidase (HRP)-labeled antibody for inoculation, antibody-antigen-enzyme-labeled antibody complex is formed. After thorough washing, the substrate TMB is added to develop color which converts to blue under the catalysis of peroxidase, and finally converts to yellow by acid termination. The depth of color is positively correlated with the content of omnipotent nuclease in the sample. The absorbance (OD value) is measured with a microplate reader at a wavelength of 450 nm, and the concentration of omnipotent nuclease in the sample is calculated according to the calibration curve.

Composition of kit

name	specification	NO.
Pre-coated Microplate	8×12 wells	1
Enzyme conjugate (100×)	0.15mL	1
Diluent	30mL	1
Chromogenic solution	6mL	2
Stop solution	6mL	1
Concentrated wash buffer (10×)	50mL	1
Concentrated standard	0.5mL	1
Plate sealer	-	4sheets
Protocol booklet	-	1copy

Storage Condition and Stability

1.Store at 2~8℃ for 12 months.

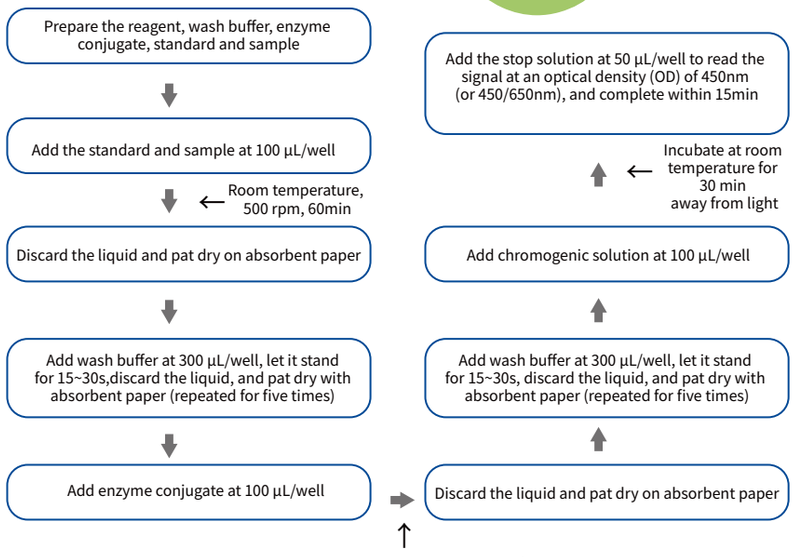
2.Please use up within 6 weeks after unpacking.

Instruments Required

1.Microplate reader (with filter at 450 nm, recommended to include dual wavelength detection mode, with primary and secondary wavelength at 450 nm and 650 nm, respectively).

2.Plate shaker (400-600 rpm).

Flow Chart



Preparation of Regents

1.Equilibrate the kit to room temperature (18~25°C).

2.Dilute 10× concentrated wash buffer with purified water at a volume ratio of 1:9 to obtain the wash working solution.

3.Dilute the 100× enzyme conjugate 100-fold with diluent before use.

4.Dilute the concentrated standard (1000 ng/mL) with diluent to 20 ng/mL, 10 ng/mL, 5 ng/mL, 2.5 ng/mL, 1.25 ng/mL, 0.63 ng/mL, 0.31 ng/mL, 0 ng/mL.

The recommended dilution method is as follows

	Final conc.	Dilution method	
NO.	(ng/mL)	Diluent	Spike amount of working standard
A	20	0.98mL	20 μL concentrated standard
B	10	0.5mL	0.5 mL A solution
C	5	0.5mL	0.5 mL B solution
D	2.5	0.5mL	0.5 mL C solution
E	1.25	0.5mL	0.5 mL D solution
F	0.63	0.5mL	0.5 mL E solution
G	0.31	0.5mL	0.5 mL F solution
H	0	0.5mL	/

Assay Procedures

1.Remove the desired strips from the aluminum foil bag equilibrated at room temperature, and seal the remaining strips in a Ziplock bag and put them back at 2-8°C.

2.Set the standard wells and sample wells. It is recommended that all standards and samples to be tested in double wells. Add 100 μL of standard at difference concentration to each standard well, and add 100 μL of sample to be tested to the sample wells.

3.Seal the reaction wells with the pleat sealer, and shake the plate (at 500 rpm) for 60min at room temperature.

4.Discard the liquid, pat dry on absorbent paper, fill each well with wash buffer (300 μL), let stand for 15~30s, shake off the wash buffer, pat dry on absorbent paper, and repeat the plate washing 5 times.

5.Add 100 μL of emzyme conjugate at working concentration to each standard well and sample well, seal the reaction wells with the plate sealer, and shake the plate (at 500 rpm) for 30min at room temperature.

6.Discard the liquid, pat dry on absorbent paper, fill each well with wash buffer (300 μL), let stand for 15~30s, shake off the wash buffer, pat dry on absorbent paper, and repeat the plate washing 5 times.

7.Add 100 μL of chromogenic solution to each well, seal the reaction wells with the plate sealer, and let stand at room temperature for 30min away from light.

8.Add 50 μL of stop solution to each well for detection within 15min, and set the wavelength of the microplate reader at 450nm (dual wavelength 450 nm/650 nm is recommended).

Interpretation of Test Results

1.The calibrated OD values of standard and samples are calculated by take the average optical density (OD) values of standard, blank control and samples, and subtracting the average OD values of the blank control. A calibration curve is plotted with the concentration of the standard as the abscissa and the OD value of calibrated standard as the ordinate. (The OD value in the dual wavelength detection mode is 450nm minus 650nm.)

2.A variety of graphing and statistical software are available to aid in the development of calibration curves and the calculation of unknown sample concentration. The cubic curve fitting method usually has a satisfactory fitting effect, and other methods such as Logistic (4P) may also achieve such a result. Therefore, applicable method needs to be analyzed according to the specific experimental data.

Test Performance

1.LOD: 0.06 ng/mL

2.LLOD: 0.2 ng/mL

3.Linearity range: 0.2~20 ng/mL

4.Precision: Intra-run CV ⩽10%, inter-run CV⩽15%

5.Recovery: 80%~120%

6.Specificity: The kit is used with Duoning Intelli Nuclease. It can also be used for detection of omnipotent nuclease from other manufacturers, but corresponding products should be used to establish a calibration curv

Cautions

1.The color development temperature and time are vital to the experimental results and should be accurately controlled.

2.During the washing process, the reaction plate should be soaked in the wash buffer for 15~30s and then dried to fully wash the non-specifically absorbed components.

3.All reagents should be shaken well before use. The spiked samples should be added to the bottom of the wells of ELISA plate to avoid adding them to the upper part of the wall. Be careful not to splash or generate bubbles.

4.If crystals are found in the concentrated wash buffer, incubate in a water bath at 37°C, and then mix and dilute to the working solution after the crystals are complete dissolved.

5.Sodium azide (NaN3) should not be added to the sample as it may destroy the activity of horseradish peroxidase, resulting in a lower detection value.

Technical Support

Please contact Duoning Biotechnology for any technical questions and supports at: marketing@duoningbio.com

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