Human Genomic DNA

H669994

Store at -20℃

Introduction:

The product is a high-purity genomic DNA extracted from 293T cells. Electrophoresis showed that the DNA was more than 15Kb in agarose gel (0.7%) without degradation, and the product was stored in TE buffer which can be widely used in PCR, enzyme digestion, hybridization, microarray analysis and other molecular biology experiments. The product was quantified with NanoDrop One at concentration of 200 ng/μL.

Contents:

Cat No.	Components	Size	Storage
H669994-20μg	Human Genomic DNA	20μg	-20℃, Avoid freeze/thaw cycle

Notes:

It is recommended to store at -20°C for a long time, and should be equilibrated to room temperature before use, and then centrifuged for use. Once the sample has been opened, it should be sealed as soon as possible.

Operation steps:

Take qPCR experiments as an example:

1. Amplification template preparation:

The samples to be tested were diluted with TE (10 mM Tris-Cl, pH 8.0, 1 mM EDTA) at a concentration between 0.05 and 10 ng/μL. Place on ice at 4°C for use.

2. Standard dilution:

Human DNA Standard 1 (100 ng/μL) was first diluted with TE to 5 standards of different concentrations according to the table below. 10 ng/μL DNA Standard 1 (Std. 1) It can be stored at -20°C for 1 month; Std. 2-5 should only be used on the same day, and should be placed at 4°C or on ice when not used.

Standards	Corresponding concentration (ng/μL)	Minimum dilution volume（Unit：μL）
Std.1	10	10 [100 ng/μL DNA Standard 1]+ 90 TE
Std.2	2.5	20 [Std. 1] +60 TE
Std.3	0.625	20 [Std. 2] +60 TE
Std.4	0.15625	20 [Std. 3] +60 TE
Std.5	0.0390625	20 [Std. 4] +60 TE

qPCR reaction system preparation:
The cryopreserved reagents required are thawed and mixed by inverting several times, and then centrifuged briefly for later use.

The base reaction of 20 μL is as follows:

Reagents	20 μL system
2×qPCR Mix	10 μL
Forward Primer, 10 μM	X μL
Reverse Primer, 10 μM	X μL
Template DNA	4 μL
ddH_2O	Up to 20 μL

Note:
High Rox model: Add 1 μL of 50×High Rox per 50 μL reaction.
Low Rox model: Add 1 μL of 50×High Rox per 500 μL reaction.

Primer concentrations of 0.2 μM are used as a reference for the set range. The concentration of the probe used is related to the fluorescence quantitative PCR instrument, the type of probe, and the type of fluorescent labeling substance.
Prepare a sufficient amount of reaction mixture as needed, and add 16 μL per well to the reaction wells after the reaction system is prepared and thoroughly mixed. The prepared standard and the diluted sample were then added to the corresponding reaction wells at an amount of 4 μL /well. TE was added to the blank control tube, also at an amount of 4 μL/well. 20μL reaction is recommended, for smaller reactions, reduce the components of the system in equal proportions.
qPCR Reaction Procedure:

Steps	Temperature	Time	Cycles
Pre-denaturation	95℃	10 min	
Denaturation	95℃	10 s	55
Annealing/Elongation	60℃	30 s	

The following is an example of the reaction conditions of our GoldStar Probe Mixture product, which should be improved and optimized according to the different PCR product templates, primer structures, and target fragment sizes used in practice.

Data analysis

Standard curve making: Draw the standard curve with reference to the data processing Excel sheet. The correlation coefficient R2 of the standard curve should not be less than 0.98, and the slope should be between -3.1 and -3.6 when the Ct value is taken as the ordinate.