BDT UltraSeq HP Kit

【Name】 BDT UltraSeq HP Kit

【Package】 YST0193-01，100rxn

YST0193-02，1000rxn

YST0193-03，5000rxn

YST0193-04，2500rxn

【Usage】

BDT UltraSeq HP Kit is a new high-performance Sanger sequencing kit. In addition to general structural DNA sequencing, it has extremely efficient sequencing function for difficult templates such as PCR products, plasmids and macromolecular DNA with hairpin structure and high GC content. After the failure of sequencing reagents from other manufacturers, BDT UltraSeq HP kit can provide a successful solution.

【Introduction】

BDT UltraSeq HP Kit is a new premixed reagent for Sanger sequencing designed for hair-pin structure DNA with high GC content. It is formulated by high-quality stable DNA polymerase and high-sensitivity four-color fluorescent dye labeled ddNTPs. It is an ideal alternative reagent for Sanger sequencing. It has better PCR reaction performance and base reading efficiency. It can obtain longer and better sequencing results on the premise of maintaining uniform peak height and optimal signal balance.

【Kit Component】

BDT UltraSeq Master Mix HP and 5x Sequencing Reaction Buffer

【Storage】

Keep reagents at - 20 ± 5 ℃, avoid repeated freezing and thawing. It can be stored for 1 year at - 20 ± 5 ℃.

【Method】

I. DNA Purity and Input of sequencing DNA template (Purity should be OD_{260}/OD_{280} = 1.6 to 2.0):

PCR products	Dosage
100-200bp	1-3ng
200-500bp	3-10ng
500-1000bp	5-20ng
1000-2000bp	10-40ng
＞2000bp	20-50ng
Single-strand DNA	25-50ng
Plasmid, double-strand DNA	150-300ng
Cosmid, BAC	0.5-1μg
DNA of bacterial genome	2-3μg

II. Preparation of reaction solution

After all reagents are taken out from the refrigerator and thaw, mix well and centrifuge before use. The preparation operation is carried out on ice. Reaction example using HP Plasmid：

Reagent	10 μL reaction system （1/8X reaction concentration）	10 μL reaction system （1/16X reaction concentration）
H_2O	5.7μL	5.8μL
HP Primer (3.2μM)	1μL	1μL
BDT UltraSeq Master Mix HP	0.5μL	0.3μL
5x Sequencing Reaction Buffer	1.8μL	1.9μL

HP Plasmid	1μL	1μL
Total volume	**10μL**	**10μL**

III. PCR reaction parameters:

Temperature	Time	Number of cycles
96 ℃	1 min	
96 ℃	10 sec	30 Cycles
50 ℃	5 sec	
60 ℃	4 min	
4 ℃	∞	

IV. Magnetic bead purification

a Prepare 85% ethanol. Take out the magnetic beads from the refrigerator and restore to room temperature.

b Swirl and mix the magnetic beads. Add 10μl magnetic beads to each sample tube.

c Add 62μl 85% ethanol into each tube, mix with a pipette for 2min.

d Place it on the magnetic stand for 5min.

e Pipette to remove the supernatant and discard.

f Add 100μl 85% ethanol and move it left and right on the magnetic stand for washing.

g After placing on the magnetic stand for 5min, pipette to remove the supernatant and discard.

h Repeat steps f and g two more times.

i Carefully remove the liquid at the bottom of the tube with a 20μl pipette and let it stand and dry for 10 ~ 20 min.

j Add 20μl ultrapure water and mix well. Wait for 5min, and then place it on the magnetic stand for 2min.

k Transfer the supernatant to a 96-well sample plate for sequencing. If you do not get on the machine immediately, please keep it frozen.

V. Capillary electrophoresis on the machine

Perform capillary electrophoresis according to the sequencing procedure of the instrument.

【Notes】

1. All relevant consumables shall be disposable to prevent cross contamination
2. Operators must receive professional training
3. Ware gloves and working clothes for protection
4. Do not use expired reagents

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