BDT UltraSeq Kit

【**Name**】 BDT UltraSeq Kit

【**Package**】 YST0181-01，100rxn

YST0181-02，1000rxn

YST0181-03，5000rxn

【**Usage**】

BDT UltraSeq Kit is mainly used in Sanger Sequencing， such as PCR products, plasmids, high molecular weight DNA templates (BCA DNA, Cosmid DNA and bacterial genomic DNA).

【**Introduction**】

BDT UltraSeq Kit is a new generation of sequencing premixed reaction solution prepared by high-quality stable DNA polymerase and high-sensitivity four-color fluorescent dye labeled ddNTP. It is an ideal alternative reagent for Sanger sequencing. It has better PCR reaction performance and base reading level. It can obtain longer and better sequencing results on the premise of maintaining uniform peak height and optimal signal balance.

【**Main component**】

BDT UltraSeq Master Mix and 5x Sequencing Reaction Buffer

【**Storage conditions and validity period**】

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

【**Method**】

I Purity and dosage of sequencing DNA template

Purity：OD_{260}/OD_{280} range from1.6 to 2.0. DNA dosage：

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 melted, please shake slightly, mix well and centrifuge before use. The preparation operation is carried out on ice, reaction system（take pGEM -3Zf(+) Control DNA as sample）：

Reagent	**10** μL reaction system （1/8X reaction concentration）	**10** μL reaction system （1/16X reaction concentration）
H_2O	5.7μL	5.8μL
-21 M13 Control Primers (3.2μM)	1μL	1μL
BDT UltraSeq Master Mix	0.5μL	0.3μL
5x Sequencing Reaction Buffer	1.8μL	1.9μL

pGEM -3Zf(+) Control (200ng/μL)	1μL	1μL
Total volume	**10μL**	**10μL**

III Sequencing of PCR reaction procedure

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, and add 10μl magnetic beads to each hole sample.

c Add 62μl 85% ethanol into each hole, blow and mix with a pipette, and place for 2 min.

d Place it on the magnetic frame for 5min.

e Pipette the supernatant and discard it.

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

g Pipette the supernatant and discard it.

h Repeat steps F and G twice, wash 3 times.

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

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

k Absorb the supernatant, transfer it to the 96 hole upper plate of the corresponding instrument, and start the sequence. If you don't 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.

【Remark】

1. All relevant consumables shall be disposable to prevent pollution and contamination.

2. The experimental personnel must receive professional training, operate in strict accordance with the instructions and strictly distinguish according to the experimental process (reagent preparation area, specimen preparation area, amplification and product analysis area). Special instruments and equipment shall be used in each stage of experimental operation, and the products used in each stage of each area shall not be used cross.

3. Protective measures shall be taken as required, such as gloves, work clothes, waste and other treatment, which shall comply with relevant national regulations.

4. Please use this reagent within the validity period.

SenseCare Medicals,Inc

425 Boulder Court,Pleasanton,CA 94566,USA

Telephone:510-861-2542

Email:SENSECARE@GMAIL.COM

Web:http://sensecarebio.com/