2×CTAB extract buffer

Item number: LS00066

Specification: 500mL

Storage: Store at room temperature, valid for 1 year.

Product Introduction:

CTAB (hexadecyltrimethylammonium bromide, hexadecyl trimethyl ammonium bromide), is a kind of cationic detergents, nucleic acid from the precipitation in low ionic strength of solution and the characteristics of acidic polysaccharides. In a high ionic strength solution (0.7mol/L NaCl), CTAB forms complexes with proteins and polysaccharides, except that it cannot precipitate nucleic acids. By extraction with organic solvent, the impurities such as protein, polysaccharide and phenols are removed, and then the nucleic acid can be separated by adding ethanol for precipitation.

How to use (for reference only) :

1. Preparation of reagents:

1. Chloroform-isoamyl alcohol mixture (24:l). Mix 96mL chloroform with 4mL isoamyl alcohol.

2. After preheating CTAB solution at 65℃, add 1mL of reducing agent (item number: M8210) into CTAB solution (mix according to 500:1 volume ratio, it is recommended to prepare according to demand, do not mix more).

2. Operation steps (for reference only) :

1. Grind about 0.1g plant tissue with liquid nitrogen, transfer to 1.5mL centrifuge tube, add 700uL 2×CTAB extract (reductant has been added). Or after the liquid nitrogen grinding, add 700uL of 2×CTAB extract into the mortar, and then absorb into 1.5mL centrifuge tube (it is recommended that the total volume should not exceed 1mL).

2. Place the centrifuge tube in a 65℃ water bath for 30min-1h, and gently shake the centrifuge tube every 10min or so during the period.

3. After the completion of the water bath, cool it for 2min, add 0.5mL of chloroform-isoamyl alcohol mixture, violently shake and mix it well. Centrifuge at 10,000-12000rpm for 5-10min.

4. Take the supernatant from the previous centrifuge tube to the new centrifuge tube.

Note: There should be three layers after the first step centrifugation: the upper layer is the water phase, the middle is the protein and plant debris, and the lower layer is the organic phase. Avoid touching the egg white and organic phase when absorbing. If accidentally absorbed into the middle and lower layer, or the middle layer is thick, it is recommended to absorb less water phase, or add 500μL chloroform-isoamyl alcohol mixture after absorption, re-mix and centrifuge to obtain supernatant.

5. Add equal volume of isopropyl alcohol into the supernatant and mix it upside-down. (or add 2 times the volume of anhydrous ethanol and 1/10 volume of 3M pH5.2 of sodium acetate).

Precipitate at -20°C for more than 30min, centrifuge at 12000rpm for l0min, and discard the supernatant.

6. The precipitation was washed with 75% ethanol once, centrifuged at 12000rmp for 3-5min, then the supernatant was discarded, dissolved in 50μL deionized water or TE buffer after precipitation and natural drying, and 1uL RNase (20μg/mL) was added and warmed at 37℃ for 30min.

7. Store at -20°C.

Alternatives:

1. Appropriate amount of PVP can be added to remove polysaccharide polyphenol pollution. PVP (polyvinylpirolidone) is a phenol complex, which can form an insoluble complex substance with polyphenols, effectively remove polyphenols and reduce phenol pollution in DNA; At the same time, it can also combine with polysaccharide, and effectively remove polysaccharide.

2. After adding CTAB extract, shake and mix, centrifuge at 5000rmp for 2min to obtain supernatant and precipitate into insufficiently ground fragments or insoluble matter. Then, chloroform-isoamyl alcohol mixture was added into the supernatant for purification, which can effectively remove the uncracked plant fragments.

3. Before adding the chloroform-isoamyl alcohol mixture, adding Tris saturated phenol (pH>7.8) -chloroform mixture to centrifuge the supernatant, and then purifying it with chloroform-isoamyl alcohol mixture, which can effectively remove the protein contamination.

Analysis of common problems:

Problems	Causes	Countermeasures
The DNA sample is impure, inhibiting the subsequent enzymatic hydrolysis and PCR reaction	DNA contains protein, polysaccharide and polyphenol impurities	Re-purify the DNA to remove impurities such as proteins, polysaccharides, and polyphenols (see previous for specific methods).
	Before the DNA is dissolved, alcohol remains, and alcohol inhibits subsequent enzymatic hydrolysis	Reprecipitate the DNA to allow the alcohol to evaporate.
	There are metal ions left in the DNA	Increase the number of washes with 70% ethanol (2-3).
	Poor experimental material or little amount	Try to use fresh (young) materials.
	Not enough wall breaking or cracking	Plants and animals should be fully homogenized and ground; G+

DNA degradation, less DNA extraction		bacteria, yeast before cracking with biological enzymes or mechanical way to break the wall; When cracking at high temperature, the time should be extended appropriately (for animal cells and bacteria, the amount of lysate can be increased).
	Incomplete precipitation	Low temperature precipitation, prolong the precipitation time; Add auxiliaries to promote precipitation.
	DNA loss during washing	When washing, it is best to use the gun head to suck out the washing liquid, do not dump.

Precautions: The reagent is easy to precipitate at low temperature, and it can be normally dissolved and used after preheating and oscillating at 50℃ for about 30min.