User guide

CTAB Buffer

CTAB Buffer
Cat. #BT02AM - 100 ml
Store at 15-25˚C

Product Description
CTAB (Cetyltrimethylammonium-bromide) based extraction is a common method for DNA purification from plant tissues. It forms insoluble complexes with nucleic acids in the presence of sodium chloride. CTAB extraction buffer effectively eliminates polysaccharides, phenolic components and other enzyme-inhibiting impurities from plant lysates.

Storage and Stability
CTAB Buffer is shipped at room temperature and stable for 2 years when stored properly.

Special Handling Instructions
CTAB Buffer contains irritants. During operation, always wear a lab coat, disposable gloves, protective goggles and procedure mask.

Materials and Equipment Required (Not Provided)

100% isopropanol [cat#BT02A]
Chloroform [cat#BT02AK]
70% ethanol [cat#BT02AH]
TE Buffer [cat#BT01AA]
Polyvinylpyrrolidone (PVP)
β-Mercaptoethanol (β-met)
Mortar and Pestle
PP 1.5 ml microcentrifuge tubes

Procedures

Immediately before use, add 2-4% Polyvinylpyrrolidone (PVP) and 0.5-2% β-Mercaptoethanol (β-met) to the required volume of CTAB buffer. It is preferred to incubate CTAB buffer at 37°C before use.

a. Tissue homogenization

1. Cut-off 50-500 mg of fresh plant tissue.
2. Add liquid nitrogen to the tissue and grind to a fine powder using a chilled mortar and pestle.

b. Tissue Lysis

1. Add 500 – 1000 µl of CTAB buffer to the tissue powder and continue grinding until becoming a homogenous solution.
2. Transfer the sample lysate into a clean 1.5ml microcentrifuge tube. Mix and thoroughly vortex.

3. Incubate the homogenate at 60°C for 30-60 minutes.
4. Centrifuge the homogenate for 10 minutes at 10,000 x g.
5. Transfer the supernatant into a new 1.5ml microcentrifuge tube.
   Optional: Add 5-10 µl of RNase (10 mg/ml) [cat# BT03AC] to lysate and incubate at 30°C for 15-20 minutes.

c. DNA Precipitation

1. Add an equal volume of chloroform: Isoamyl alcohol (24:1) to the lysate. Vortex for 5 seconds then centrifuge for a minute at 14,000 x g to separate the phases.
2. Transfer the upper aqueous phase to a clean 1.5ml microcentrifuge tube.
3. Precipitate the DNA by adding 0.7 volumes of ice-cold isopropanol. Mix by inversion gently and incubate at -20°C for 15 minutes.
4. Centrifuge for 10 minutes at 14,000 x g. Decant the supernatant without disturbing the pellet and wash the pellet with 500 µl ice-cold 70% ethanol. Invert the tube gently.
5. Centrifuge for 1 minute at maximum speed. Remove ethanol.
6. Air-dry the pellet at room temperature or 37°C. Avoid DNA pellet over-drying.
7. Resuspend the DNA in 50-100 µl of TE buffer or nuclease-free water.
   Optional: pre-heat TE buffer or nuclease-free water at 37°C.

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