



BIOLOGICAL INDUSTRIES
ISRAEL BEIT HAEMEK LTD
Kibbutz Beit Haemek 25115 Israel
Tel: 04-9960595 Fax: 04-9968896
E-mail: info@bioind.com Website: www.bioind.com



EZ-DNA

Genomic DNA Isolation Reagent

Cat. No.: 20-600-50
Store at: Room Temperature

Product Description

EZ-DNA is a non-organic and ready to use reagent for the isolation of genomic DNA from samples of human, animal, plant, yeast, bacterial and viral origin. **EZ-DNA** is an improved version of the Chomczynski method (1), which is based on disruption of cells in a guanidine-detergent lysing solution that hydrolyzes RNA and allows the selective precipitation of DNA from a cell lysate with ethanol. Following an ethanol wash, DNA is solubilized in water or 8 mM NaOH. There is no phenol in **EZ-DNA**. The protocol is fast and permits isolation of genomic DNA from a large number of samples of small or large volumes. The procedure can be completed in 10-30 minutes with DNA recovery of 70-100%. The isolated DNA can be used, without additional purification, for southern analysis, dot blot hybridization, molecular cloning, RFLP, PCR and other molecular biology and biotechnology applications.

Kit Reagent

50ml solution containing Guanidinium Isothiocyanate.

Reagent Required But Not Supplied

- * Absolute Ethanol
- * 95% Ethanol
- * 8 mM NaOH (fresh preparation)
- * Chloroform (for plants)
- * Digestion Buffer (for mouse tail): 50mM Tris pH 8.0
100mM EDTA
0.5% SDS
- * RBC Lysis Solution, Cat. No. 01-888-1 (for whole blood)

Storage

EZ-DNA should be stored at room temperature. However, storing at lower temperatures will cause the guanidine isothiocyanate to come out of the solution. If the reagent is warmed, the guanidine isothiocyanate should resolubilize instantly.

Handling Precautions

EZ-DNA contains irritants. Handle with care, avoid contact with skin, and use eye protection. In case of contact, wash skin with a large amount of water. Seek medical attention.

Protocol for Genomic DNA Isolation

1. Homogenization

All samples should be gently, but thoroughly, homogenized with the EZ-DNA reagent. Homogenization can be achieved by repetitive pipetting with a Pasteur pipette. The sample will become viscous due to the release of high molecular weight genomic DNA. Do not pipette the sample too vigorously, as this will shear the genomic DNA. All samples should be held at room temperature for 5 minutes, unless stated otherwise.

1.1 Tissue

Gently homogenize tissue samples in the reagent. Use 1ml EZ-DNA per 50mg tissue.

1.2 Cells

Cells grown in monolayer - should be lysed directly in the culture dish by addition of 1ml EZ-DNA per 10cm² area of culture dish. Discard the media, add EZ-DNA and pass the cell lysate several times through a pipette.

Cells grown in suspension - use 1ml EZ-DNA per 10⁷ cells. The cells should be pelleted and then lysed. Alternatively, use the suspension (volume < 0.1ml).

Cell nuclei - use 1ml EZ-DNA per 10⁷ cell nuclei. The nuclei can be either in pellet or suspension (volume < 0.1ml). Mix the samples by inverting the tubes or repeated pipetting.

1.3 Bacterial Cells

gram positive - use 1ml EZ-DNA per 10⁷ cells. Freeze cells in liquid nitrogen and grind to a fine powder using a mortar and pestle, homogenize briefly, and gently mix for 1 hour at 60°C.

gram negative - sediment the cells and use 1ml EZ-DNA per 10⁷ cells. Lyse the cells by repetitive pipetting and gently mix for 15-60 minutes at 60°C.

1.4 Yeast Cells

Sediment the cells and use 1ml EZ-DNA per 10⁷ cells. Homogenize briefly and gently mix for 15-60 minutes at 60°C.

1.5 Plant

Use 1ml EZ-DNA per 50-200mg of plant. Freeze cells in liquid nitrogen and grind to a fine powder using a mortar and pestle or homogenizer. Gently mix for 1 hr at 60°C, and proceed with section 2 of the protocol. For plant tissues containing polyphenolics, use EZ-Plant (Cat. No. 01-893-1) to assist with DNA isolation.

1.6 Liquid Matrices

To isolate DNA from liquid matrices including stool, sputum, urine, wound exudate and viral cultures, gently homogenize 1ml sample in 10-15ml EZ-DNA.

1.7 Whole Blood

To isolate DNA from fresh whole blood, add 1ml of whole blood to 2ml RBC Lysis Solution (Cat. No. 01-888-1). Gently mix at room temperature for 5 to 10 minutes. Centrifuge at 300g for 10 minutes and discard the supernatant. Add 1ml EZ-DNA, and mix the sample by repeated pipetting. Hold for 5 minutes at room temperature. To isolate DNA from frozen blood, use EZ-Blood buffer solution (Cat. No. 01-894-1) for nuclei isolation prior to DNA extraction.

1.8 Mouse Tail

Add pieces (1-3mm) of mouse tail (up to 20mg) to 0.5ml digestion buffer supplemented with 400µg/ml proteinase K. Incubate at 55°C for 1-4 hours with mixing, or overnight at room temperature. Briefly centrifuge the samples and transfer the supernatant to a new tube. Add 0.5ml EZ-DNA to the supernatant and incubate at room temperature for 5 minutes. Add 1ml of absolute ethanol, mix, and allow to sit for 1-3 minutes. Spool the DNA and proceed with Step 5.

1.9 Biohazardous Material

When working with biohazardous material, proteinase K digest can be used (see 1.8). This technique eliminates aerosols and improves biosafety.

2. Phase Separation (PLANTS ONLY)

Add 1ml chloroform per 1ml EZ-DNA. Allow to stand for 5 minutes at room temperature, and centrifuge at 12,000g for 10 minutes at room temperature. Following centrifugation, transfer the upper (aqueous) phase to a clean tube and precipitate the DNA by adding ethanol: 1 volume of aqueous phase with 1 volume of ethanol. Mix the samples by inverting the tubes 10 times and store them at room temperature for 5 minutes. Sediment precipitated DNA at 5,000g for 4 minutes and discard the resulting supernatant.

3. Centrifugation (optional)

This step removes insoluble tissue fragments, and is recommended for tissues containing a large amount of extracellular material (liver, muscle). Centrifuge at 10,000g for 10 minutes at room temperature.

4. DNA Precipitation

Add 1ml of absolute ethanol per 1ml of EZ-DNA. Mix samples by inverting the tubes 10 times. Make sure that the EZ-DNA and the ethanol make a homogenous solution. Store the samples for 3 minutes at room temperature. DNA should become visible. Remove the DNA by spooling with a pipette tip or centrifuge at 5,000g for 5 minutes. For small quantities of DNA use centrifugation.

5. DNA Wash

Wash the DNA pellet twice with 1ml 95% ethanol. To remove contaminants from difficult sources (such as liver, kidney, yeast, gram positive bacteria), for the first wash use solution containing 50% EZ-DNA and 50% ethanol. Suspend the DNA by inverting the tubes 10 times. Allow the DNA to settle to the bottom or centrifuge at 1,000g for 1 minute. Remove the ethanol.

6. DNA Solubilization

Remove remaining ethanol wash and air-dry the DNA pellet for 5 minutes. Do not let the DNA pellet dry completely. Dissolve the DNA in 8 mM NaOH (fresh preparation). Add a sufficient amount to reach your desired concentration. Note that a higher concentration than 0.3µg/µl will cause a very viscous solution that will be hard to work with. Store the sample for 5 minutes and then dissolve the DNA by pipetting. For high concentrations, heating at 55°C will be required. For preparation from tissues or plants containing insoluble material, remove the insoluble material by centrifugation at 12,000g for 10 minutes. The final preparation of genomic DNA isolated with EZ-DNA contains 20-100 kb with the A_{260}/A_{280} at a ratio of 1.6-1.9.

Note

The resulting DNA may contain some degraded RNA. The concentration of the RNA is less than 3% of the DNA. For most methods this is no problem. If you require RNA-free DNA, apply RNase to the DNA sample.

pH Adjustment of DNA Samples Dissolved in 8mM NaOH

For 1ml of 8mM NaOH, use the following amounts of 1M Hepes, free acid:

Final pH	1M Hepes (µl)
7.0	42
7.2	30
7.5	18
7.8	13.5
8.0	11.5
8.4	9.5

Halting Points During Isolation

- * The lysate which contains EZ-DNA can be stored for: 18 hours at room temperature
9 months at 4°C
9 months at -20°C
- * During washes, DNA can be stored in 95% ethanol for at least 1 week at room temperature, or 3 months at 4°C.
- * For long-term storage of high molecular DNA, re-precipitate the DNA and store in ethanol at 4°C.

Reference

- (1) Chomczynski, P. and Sacchi, N., *Anal Biochem.*, **162**:156-159 (1987)

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EZ-DNA

Step	Action	Remarks
Lysis \ Homogenization		
<i>tissue, cells, liquid material</i>	1ml EZ-DNA + 50mg tissue, 10 ⁷ cells or 0.1ml liquid material	incubate for 5 minutes at room temperature
<i>mouse tail</i>	20mg mouse tail + 0.5ml digestion buffer supplemented with 400µg/ml proteinase K lysate + 0.5ml EZ-DNA	Incubate for 1-4 hours at 55°C, centrifuge to remove undigested material 5 minutes at room temperature
<i>whole blood</i>	1ml whole blood + 2ml RBC lysis solution leukocyte pellet + 1ml EZ- DNA	5 minutes at room temperature, centrifuge at 300g for 5minutes 5 minutes at room temperature
<i>plant tissue</i>	50-200mg plant tissue + 1ml EZ-DNA lysate + 1ml chloroform	mix for 1 hour at 60°C 5 minutes at room temperature, centrifuge at 12000g for 10minutes
Centrifugation (optional)		10,000g * 10 minutes
DNA Precipitation	lysate + 1ml absolute ethanol	mix well, store for 3 minutes at room temperature
DNA Wash	1ml 95% ethanol (*2).	mix well, store for 2 minutes
DNA Solubilization	8 mM NaOH or water	heat if necessary