EZ-DNA

Genomic DNA Isolation Reagent

Cat. No.: 20-600-50

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BIOLOGICAL INDUSTRIES
ISRAEL, BEIT HAEMEK LTD.

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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 nstantly.

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 $10cm^2$ 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 10^7 cells. The cells should be pelleted and then lysed. Alternatively, use the suspension (volume < 0.1ml).
Cell nuclei - use 1ml EZ-DNA per 10^7 cell nuclei. The nuclei can be either
Mix the samples by inverting the tubes or repeated pipetting.

1.3 Bacterial Cells

gram positive - use 1ml EZ-DNA per 10^7 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 10^7 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 10^7 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 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 400 μg/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 0.3mg/ml 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)

Tissue Specific Recommendations for the Use of EZ-DNA

Tissue/Sample Type	Recommendations
Tissues	Add 50mg of mammalian tissue (either fresh or frozen at -70°C until use) to 1ml EZ-DNA. Homogenize the tissue with few strokes as possible, so as not to shear the DNA. The method of homogenization will affect the size and yield of the DNA. Soft tissue, such as brain, does not require the use of a homogenizer: a pipette can be used to homogenize. Centrifugation of the homogenate at 12,000g for 10 minutes prior to the addition of ethanol is a mandatory step for some tissues, such as liver, muscle. Use of thawed samples is not recommended.
Cell Cultures	**Suspension Culture** - Cells should be collected by centrifugation. Discard the medium and add 1ml EZ-DNA per 10^7 cells. If the medium is not acidic, it is possible to add the cells directly to the EZ-DNA without centrifugation. The sample volume should be <0.1 volume of EZ-DNA. **Monolayer Culture** - The medium should be decanted before adding the EZ-DNA directly to the plate. Do not wash the cells. Add 1ml EZ-DNA per 10 cm^2. Allow to sit for a minute and transfer to a tube. For hard types of cells, extend the incubation and scrape off the cells. Proceed with the protocol.
Epithelium Cells	Epithelium cells should be collected by centrifugation (5000rpm for 3 minutes). Remove the supernatant and re-suspend the cell pellet (107 cells) in 1ml EZ-DNA.
Plants	Pulverize 100mg of plant tissue in liquid nitrogen with mortar and pestle or homogenizer. Place the powder in a microcentrifuge tube and resuspended with 1ml EZ-DNA. Mix well and incubate for 15-60 minutes at 60°C with gentle mixing. Centrifugation of the lysate prior to the addition of chloroform is a mandatory step. Add 1ml of chloroform per 1ml EZ-DNA, mix well and store the sample for 10 minutes at room temperature. Centrifuge the sample at 12,000g for 10 minutes. The DNA is in the upper aqueous phase. Very carefully collect the aqueous phase and transfer to a new tube. Proceed with the protocol. Use of thawed samples is not recommended. For plant tissues containing polyphenolic compounds-the use of EZ-Plant (Cat. No. 01-893-1) is recommended.

Tissue/Sample Type	Recommendations
Blood	Whole blood must be collected in EDTA or citrate-coated tubes to prevent clotting and DNA degradation. **DNA extracted from heparinised blood cannot be used for PCR.** Add 1ml of whole blood to 2ml RBC Lysis Solution (Cat. No. 01-888-1). Allow to sit for 5-10 minutes at room temperature with gentle mixing. Centrifuge at 300g for 10 minutes and discard the supernatant. Repeat process if red blood cells are evident in the pellet. Typically add 1ml EZ-DNA to the leukocyte pellet (add 1ml EZ-DNA per 1ml whole blood). Mix well and proceed with the rest of the protocol. To isolate DNA from frozen blood, use EZ-Blood (Cat. No.01-894-1). The procedure includes a nuclear isolation step prior to DNA extraction.
Mouse Tail	Add small pieces (1-3mm) of mouse tail (up to 20mg) to a tube containing 0.5ml digestion buffer (50mM Tris pH8, 100mM EDTA, 0.5% SDS) supplemented with 400μg/ml proteinase K. Incubate at 55°C for 1-4 hours with mixing, or overnight at room temperature for complete digestion of the tail pieces. Add 0.5ml EZ-DNA and allow to sit for 5 minutes. Add 1ml of absolute ethanol, mix, and allow to sit for 1-3 minutes. Spool the DNA and wash twice with 95% ethanol. Remove the ethanol and re-suspend with 8mM NaOH.
Bacterial Cultures	**Gram negative** - Add 1ml EZ-DNA per 10^7 cells. Lyse the cells by repetitive pipetting and incubate at 60°C for 15-60 minutes. Add 1ml absolute ethanol per 1ml EZ-DNA. Precipitate with centrifugation at 5000g for 4 minutes. Wash twice with 1ml 95% ethanol. Centrifuge at 5000g for 4 minutes, remove the ethanol and store the tubes for 5 minutes. Add 8mM NaOH to dissolve the DNA. Use cells in stationary phase. **Gram positive** - 10^7 cells should be pulverized in liquid nitrogen with mortar and pestle or homogenizer. Place the powder in a microcentrifuge tube and resuspend with 1ml EZ-DNA. Mix well and incubate for 15-60 minutes at 60°C with gentle mixing. Add 1ml absolute ethanol per 1ml EZ-DNA. Precipitate with centrifugation at 5000g for 4 minutes. Wash with 1ml solution containing 50% EZ-DNA and 50% ethanol. Centrifuge at 5000g for 4 minutes. Wash with 1ml 95% ethanol. Centrifuge at 5000g for 4 minutes, remove the ethanol and store the tubes for 5 minutes. Add 8mM NaOH to dissolve the DNA. Use cells in stationary phase.

Yeast	Pellet 10^7 cells and add 1ml of EZ-DNA. Lyse the cells by repetitive pippeting with mortar and pestle or homogenizer. Incubate the sample for 15-60 minutes at 60°C with gentle mixing. Add 1ml absolute ethanol per 1ml EZ-DNA. Precipitate with centrifugation at 5000g for 4 minutes. Wash with 1ml solution containing 50% EZ-DNA and 50% ethanol. Centrifuge at 5000g for 4 minutes. Wash with 1ml 95% ethanol. Centrifuge at 5000g for 4 minutes, remove the ethanol and store the tubes for 5 minutes. Add 8mM NaOH to dissolve the DNA.
Biohazardous Material	Add 20mg tissue to a tube containing 0.5ml digestion buffer (50mM Tris pH8, 100mM EDTA, 0.5% SDS) supplemented with 400mg/ml proteinase K. Incubate at 55°C for 1-4 hours with mixing, or overnight at room temperature for complete digestion of the tail pieces. Add 0.5ml EZ-DNA and allow to sit for 5 minutes. Add 1ml of absolute ethanol, mix, and allow to sit for 1-3 minutes. Spool the DNA and wash twice with 95% ethanol. Remove the ethanol and re-suspend with 8mM NaOH.

Assessing Yield of Genomic DNA

The yield of genomic DNA will vary depending on the tissue or cells from which it is obtained.

Tissue/Sample Type	Amount of Starting Material	Yield of Genomic DNA
Liver	1mg	3-4μg
Kidney	1mg	3-4μg
Skeletal Muscle	1mg	2-3μg
Brain	1mg	2-3μg
Placenta	1mg	2-3μg
Human Cells	10^6 cells	5-7μg
Rat Cells	10^6 cells	5-7μg
Mouse Cells	10^6 cells	5-7μg
Lung	1mg	3-5μg
Heart	1mg	2-3μg
Plant Leaf	1gr	20-200μg
Whole Blood	1ml	20-40μg

Sf9 Cells	10^7 cells	170-180μg
E.coli Cells	10^9 cells	30-40μg
Mouse Tail	1mg	0.4-3μg

Troubleshooting Guide: DNA Isolation with EZ-DNA

Problem	Sample	Possible Cause	Suggested Solution
Low Yield of DNA	*Any sample*	Incomplete homogenization of samples	Make sure no particulate matter remains. Be sure to incubate for 5 minutes at room temperature after homogenization.
		Insufficient volume of ethanol during precipitation	Add ethanol up to 50% of final volume.
		DNA pellet was not completely dissolved	Precipitate the DNA with ethanol and dissolve the pellet using a larger volume of 8 mM NaOH. Heat the sample at 55^{0}C for 10 minutes.
	Blood	Sample was stored at 2-4^{0}C longer than 2 months; Blood clots were present in the sample	Take new sample.
	Cell culture	Too many cells were used for purification, and DNA pellet turned insoluble;	Reduce cell quantity twice or more. Take new sample.
Degraded DNA	*Any sample*	Samples were homogenized too vigorously, resulting in shearing of the DNA	Avoid high speed homogenizers.

Problem	Sample	Possible Cause	Suggested Solution
		Cell/tissue was not processed or frozen immediately after removing from animal or culture	Process the cell/tissue immediately after removing from the culture or the animal.
	Mouse tail	Digestion was too long	Shorten the incubation time at 55°C. Do not incubate overnight.
RNA Contamination	*Any sample*	pH change	Be sure to remove as much sample medium as possible from the cell pellet. Add RNase A at a final concentration of 0.2mg/ml to the sample prior to the addition of ethanol, and incubate for 10 minutes at 37°C.
	Plant	The upper aqueous phase was removed with some of the organic phase	Remove the upper aqueous phase very carefully without the organic phase.
Low A_{260}/A_{280} Ratio	*Any sample*	Insufficient washing of precipitated DNA	For the first wash use a solution containing 50% EZ-DNA and 50% ethanol. Alternatively, wash one more time with 95% ethanol.
		Acidic pH of the water used for the solubilization	Use alkaline solution 8 mM NaOH, or phosphate buffer for the spectro-photometer
Enzymatic Reactions not Running	*Any sample*	Residual reagents (ethanol, salts, etc.) present in prepared DNA	Precipitate DNA with ethanol, wash with cold 70% ethanol and dissolve in sterile de-ionized water.
	Blood	Heparinised blood was used.	Use EDTA or citrate treated blood.

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EZ-DNA

Step	Action	Remarks
Lysis \ Homogenization		
tissue, cells, liquid material	1ml EZ-DNA + 50mg tissue, 10^7 cells or 0.1ml liquid material	incubate for 5 minutes at room temperature
mouse tail	20mg mouse tail + 0.5ml digestion buffer supplemented with 400 μg/ml proteinase K	Incubate for 1-4 hours at 55°C, centrifuge to remove undigested material
	lysate + 0.5ml EZ-DNA	5 minutes at room temperature
whole blood	1ml whole blood (fresh) + 2ml RBC lysis solution	5 minutes at room temperature, centrifuge at 300g for 5minutes
	leukocyte pellet + 1ml EZ-DNA	5 minutes at room temperature
plant tissue	50-200mg plant tissue + 1ml EZ-DNA	mix for 1 hour at 60°C
	lysate + 1ml chloroform	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 (x2)	mix well, store for 2 minutes
DNA Solubilization	8 mM NaOH or water	heat if necessary

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