DNA STAT

DNA STAT-60™

SINGLE REAGENT FOR RAPID DNA ISOLATION

Cat. No. TL-4200 | For in vitro research use only | TEL-TEST Bulletin No. 10

1. INTRODUCTION:

DNA STAT-60, a single monophasic reagent containing a chaotropic cell disrupter and a non-corrosive phenol free extraction reagent, replaces cumbersome, labor intensive methods of genomic DNA isolation. Following tissue or cell homogenization in the DNA STAT-60, and after the addition of chloroform, the homogenate separates into two phases: the aqueous phase and organic phase. The DNA remains in the aqueous phase while RNA and the other cellular components, including proteins, are preferentially partitioned in the organic phase and interface. The DNA STAT-60 method does not require ultracentrifugation, and can be completed in under 1 hour. The DNA STAT-60 isolates high molecular weight genomic DNA from samples of human, animal, plant, yeast, and bacterial origin and is particularly well suited for the simultaneous processing of multiple samples.

RNA and proteins are sequestered in organic and interface: DNA remains in upper aqueous phase.

Protocol can be completed in 60 minutes.

Single extraction step replaces cumbersome, time and labor intensive steps requiring proteinase K, phenol, or solid supports.

Single monophasic reagent with extended shelf life.

Does not contain phenol or hazardous organics.

Isolates genomic DNA from samples of human, animal, plant, yeast and bacterial origin. Can be used to back extract DNA from "SINGLE STEP METHOD" RNA preps (i. e. RNAzol B. RNA STAT-60).

2. REAGENTS SUPPLIED:

DNA STAT-60™	50 ml, 100 ml, or 200 ml bottle containing clear solution of DNA STAT-60™.
PREPARATION:	Ready to use
STORAGE:	Refrigerate at 2-8°C. Protect from exposure to light.
STABILITY:	6 months. Refer to expiration date stamped on label.

3. REAGENTS REQUIRED, BUT NOT SUPPLIED:

Chloroform (ACS grade)
Isopropanol (ACS grade)
Ethanol (ACS grade)

4. PROTOCOL

DNA isolation by the DNA STAT-60™ method includes the following steps:

1. Homogenization	DNA STAT-60 (1 ml per 50-100 mg tissue, or 5-10 x 10^6 cells).
2. DNA Extraction	1 vol. of homogenate + 0.2 vol. of chloroform
3. DNA Precipitation	0.5 vol. of isopropanol
4. DNA Wash	75% ethanol

Unless stated otherwise the procedure is carried out at room temperature.

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4.1 HOMOGENIZATION

A. TISSUES:

Homogenize tissues in the DNA STAT-60 (1 ml/50-100 mg issue) in a glass-teflon or polytron homogenizer.

B. CELLS:

Cells grown in monolayer are lysed directly in a culture dish by adding DNA STAT-60 (1 ml/3.5 cm petri dish) and passing cell lysate 5-10 times through a pipette. Cells grown in suspension are sedimented then lysed in a DNA STAT-60 (1 ml per 5-10x10^6 cells) by repetitive pipetting.

4.2 DNA EXTRACTION:

Following homogenization, add 0.2 ml of chloroform per 1 ml of DNA STAT-60, cover the sample tightly, shake vigorously for 15 seconds and let it stay at room temperature for 2-3 minutes. Centrifuge the homogenate at 12,000g(max) for 15 minutes at 4°C. Following centrifugation the homogenate separates into two phases: a lower organic phase and the upper aqueous phase. DNA remains in the aqueous phase whereas RNA and proteins are in the interface and the organic phase.

4.3 DNA PRECIPITATION

Transfer the aqueous phase to a fresh tube and mix with isopropanol. Add 0.5 ml of isopropanol per 1 ml of the DNA STAT-60 used for homogenization. Store samples at room temperature for 5-10 minutes and centrifuge at 12,000g(max) for 10 minutes at 4°C. The DNA precipitate forms a small clear to white pellet at the bottom of the tube.

4.4 DNA WASH

Remove supernatant and wash the DNA pellet once with 75% ethanol by vortexing and subsequent centrifugation at 7,500g(max) for 5 minutes at 4°C. Add at least 1 ml of 75% ethanol per 1 ml of the DNA STAT-60 used for the initial homogenization.

At the end of the procedure, dry the DNA pellet briefly by air drying (5-10 min.) Dissolve the DNA pellet in water or in 1 mM EDTA, pH 7. Pass the pellet a few times through a pipette tip. An incubation for 10-15 minutes at 55-60°C may be required to dissolve DNA samples.

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5. RECOVERING DNA FROM "SINGLE STEP METHOD" (RNAzol B, RNA STAT-60 PREPS)

5.1 DNA REVERSE EXTRACTION

Remove aqueous layer containing RNA. Add 800 ul of DNA STAT-60 reagent per 1 ml of RNAzol B or RNA STAT-60 used for the initial homogenization. Add 0.2 ml of chloroform per 1 ml of DNA STAT-60, shake vigorously for 15 seconds and let it stay at room temperature for 2-3 minutes. Centrifuge the homogenate at 1000g(min)-12,000g(max) for 15 minutes at 4°C. Following centrifugation the homogenate separates into two phases: a lower organic phase and the upper aqueous phase. DNA remains in the aqueous phase whereas RNA and proteins are in the interface and organic phase.

5.2 DNA PRECIPITATION

Follow procedure as outlined in section 4.3.

5.3 DNA WASH

Follow procedure as outlined in section 4.4.

6. SPECIAL HANDLING PRECAUTIONS

The DNA STAT-60 contains an irritant (Guanidinium Salts) Can be fatal. When working with DNA STAT-60 use gloves and eye protection (shield, safety goggles). Do not get on skin or clothing. Avoid breathing vapor. Read warning note on bottle.

REFERENCES

1. Chomczynski, P. and Sacchi, 1987. Single-Step Method and RNA Isolation by Acid Guanidinium Thiocyanate-Phenol-Chloroform Extraction. Biotechniques 8:148-149.

2. Maniatis, T., E.T. Fritsch and J. Sambrook, 1982. Molecular Cloning: A Laboratory Manual.

3. Winberg, G., 1991. A Rapid Method for Preparing DNA from Blood. Suited for PCR Screening of Transgenes in Mice. PCR Methods Applic. 1::72-74.

DNA STAT-60™

CAT. NO. TL-4200 50 ml
CAT. NO. TL-4210 100 ml
CAT. NO. TL-4220 200 ml