Promega

Technical Bulletin

ReadyAmp™ Genomic DNA Purification System

INSTRUCTIONS FOR USE OF PRODUCTS A7710.

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Part# TB190

ReadyAmp™ Genomic DNA Purification System

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1. Description

The ReadyAmp™ Genomic DNA Purification System provides a simple, effective, safe and inexpensive approach to isolate single-stranded genomic DNA from whole blood or bloodstains for amplification analysis. The process takes less than one hour and requires no organic extractions or ethanol precipitations. The ReadyAmp™ Genomic DNA Purification System produces single-stranded DNA (ssDNA) that may be used directly in amplification reactions and PCR without further manipulation.

Note: The ReadyAmp™ Genomic DNA Purification System was designed and optimized for the isolation of ssDNA for use in amplification procedures. For applications that require double-stranded plasmid DNA, lambda DNA or smaller DNA fragments, we highly recommend the Wizard® DNA Purification Systems.

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2. Product Components and Storage Conditions

Product	Size	Cat. #
ReadyAmp™ Genomic DNA Purification System	100 preps	A7710

Each system contains sufficient reagents for 100 samples. Includes:

- 20ml ReadyAmp™ Genomic DNA Purification Resin
- 100ml Nuclease-Free Water (4 × 25ml)
- 1 Autoclaved Magnetic Stir Bar

Storage and Stability: Store at room temperature.

3. Genomic DNA Purification Protocols

Materials to Be Supplied by the User

- 1.5ml microcentrifuge tubes
- 56°C water bath or heating block
- 100°C water bath or heating block

3.A. DNA Purification from Whole Blood

Before beginning this protocol, preheat one water bath or heating block to 56°C and a second water bath or heating block to 100°C.

1. Transfer 1ml of Nuclease-Free Water into labeled 1.5ml microcentrifuge tubes.
2. Add 1–400µl of whole blood to each tube and vortex for 5–10 seconds.
3. Incubate at room temperature for 10 minutes. Vortex the sample(s) every 1–2 minutes during this incubation.
4. Centrifuge the sample(s) at top speed (15,000rpm) for 2 minutes at room temperature in a microcentrifuge.
5. Remove and discard the supernatant without disturbing the pellet.
6. For the first use of the system, carefully tip the Autoclaved Magnetic Stir Bar into the bottle of ReadyAmp™ Resin. Cover the bottle of ReadyAmp™ Resin and vigorously stir the suspension on a magnetic stir plate.

   Note: Remove aliquots of ReadyAmp™ Resin while the resin is stirring.

7. Add 200µl of resuspended resin to each sample.

   Note: When the resin volume falls below 5ml, swirl the bottle immediately before use to ensure that the resin has been resuspended.

8. Vortex the sample(s) to resuspend the pellet(s). **Make certain that the entire pellet has been resuspended.**
9. Incubate the sample(s) for 20 minutes in a 56°C water bath or heating block.

10. Vortex at high speed for 5–10 seconds.

11. Incubate the sample(s) for 8 minutes in a 100°C water bath or heating block.

12. Vortex at high speed for 5–10 seconds.

13. Centrifuge the sample(s) at top speed (15,000rpm) for 2 minutes at room temperature in a microcentrifuge.

    The isolated genomic ssDNA is in the supernatant. The DNA sample(s) may be stored at 4°C or –20°C or used directly in applications including PCR amplification.

Notes:

1. 1–5µl of sample is generally sufficient template for a 50µl amplification reaction, depending on the amount of starting material.

2. If the DNA samples have been stored, repeat the centrifugation in Step 13 before use. The yield of DNA may be estimated by rapid slot blot detection, using dilutions of a known quantity of genomic DNA for comparison (1).

Table 1. Relationship Between Starting Volume and Expected Yield.

Starting Volume of Blood (µl)	Expected ssDNA Yield (µg)
1	0.04–0.06
10	0.2–0.4
100	2–4
200	4–6
400	8–10

3.B. DNA Purification from Bloodstains

Before beginning this protocol, preheat one water bath or heating block to 56°C and a second water bath or heating block to 100°C.

1. Transfer 1ml of Nuclease-Free Water into labeled 1.5ml microcentrifuge tubes.

2. Add a 9–25mm^2 piece of bloodstained material to each tube.

3. Incubate at room temperature for 10 minutes. Vortex the sample(s) every 1–2 minutes during this incubation.

4. Centrifuge the sample(s) at top speed (15,000rpm) for 2 minutes at room temperature in a microcentrifuge.

5. Remove and discard the supernatant. Leave the bloodstained material in the tube with the pellet.

6. For the first use of the system, carefully tip the Autoclaved Magnetic Stir Bar into the bottle of ReadyAmp™ Resin. Cover the bottle of ReadyAmp™ Resin and vigorously stir the suspension on a magnetic stir plate.

   Note: Remove aliquots of ReadyAmp™ Resin while the resin is stirring.

7. Add 200µl of resuspended resin to each sample.

   Note: When the resin volume falls below 5ml, swirl the bottle immediately before use to ensure that the resin has been resuspended.

8. Vortex the sample(s) to resuspend the pellet. **Make certain that the entire pellet has been resuspended.**

9. Incubate the sample(s) for 20 minutes in a 56°C water bath or heating block.

10. Vortex at high speed for 5–10 seconds.

11. Incubate the sample(s) for 8 minutes in a 100°C water bath or heating block.

12. Vortex at high speed for 5–10 seconds.

13. Centrifuge the sample(s) at top speed (15,000rpm) for 2 minutes at room temperature in a microcentrifuge.

    The isolated genomic ssDNA is in the supernatant. The DNA sample(s) may be stored at 4°C or –20°C or used directly in applications including PCR amplification.

Notes:

1. 1–5µl of sample is generally sufficient template for a 50µl amplification reaction, depending on the amount of starting material.

2. If the DNA samples have been stored, repeat the centrifugation in Step 13 before use. The yield of DNA may be estimated by rapid slot blot detection, using dilutions of a known quantity of genomic DNA for comparison (1).

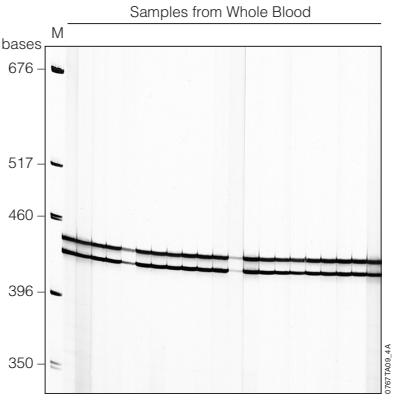

Figure 1. Amplification analysis of DNA isolated from whole blood using the ReadyAmp™ Genomic DNA Purification System. Genomic ssDNA was isolated from 21 whole blood samples (100µl each) following the protocol in Section 3.A. A 5µl portion of each 200µl ssDNA preparation was amplified at the D1S80 locus. Amplification products were separated in a 4% denaturing polyacrylamide gel and detected by silver stain analysis. Lane M: pGEM® DNA Markers (Cat.# G1741).

4. Related Products

Product	Size	Cat.#
Wizard® PCR Preps DNA Purification System	50 preps	A7170
Wizard® PCR Preps DNA Purification Resin	250ml	A7181
Wizard® Genomic DNA Purification Kit	100 × 300µl	A1120
	500 × 300µl	A1125
Cell Lysis Solution	1L	A7933
Nuclei Lysis Solution	50ml	A7941
	1L	A7943
Protein Precipitation Solution	25ml	A7951
	350ml	A7953
DNA Rehydration Solution	50ml	A7963
RNase A Solution, 4mg/ml	1ml	A7973
pGEM®-T Vector System I	20 reactions	A3600
pGEM®-T Vector System II	20 reactions	A3610
pGEM®-T Easy Vector System I	20 reactions	A1360
pGEM®-T Easy Vector System II	20 reactions	A1380
dATP, dCTP, dGTP, dTTP	40µmol each	U1240

For Laboratory Use

5. Reference

1. Sequences Application Update #371, Schleicher & Schuell, Inc.

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All prices and specifications are subject to change without prior notice.

Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.

This quick protocol is intended as an easy-to-follow reminder for experienced users. Please follow the complete protocol (Technical Bulletin #TB190) the first time you use the ReadyAmp™ Genomic DNA Purification System.

DNA Purification from Whole Blood

1. Transfer 1ml of Nuclease-Free Water into 1.5ml tube(s).
2. Add 1–400µl of whole blood to each tube(s) and vortex for 5–10 seconds.
3. Incubate at room temperature for 10 minutes, vortexing every 1–2 minutes.
4. Centrifuge at top speed for 2 minutes and discard the supernatant. Do not disturb the pellet.
5. Place the autoclaved magnetic stir bar in the bottle of ReadyAmp™ Resin and vigorously stir the suspension.
6. While the resin is stirring, remove 200µl aliquots of the resuspended resin to each tube.
7. Vortex the sample(s) to resuspend the pellets.
8. Incubate the sample(s) for 20 minutes in a 56°C water bath or heating block and then vortex for 5–10 seconds.
9. Incubate the sample(s) for 8 minutes in a 100°C water bath or heating block and then vortex for 5–10 seconds.
10. Centrifuge the sample(s) at top speed for 2 minutes. The isolated ssDNA will be in the supernatant. Store at 4°C or –20°C.

DNA Purification from Bloodstains

1. Transfer 1ml of Nuclease-Free Water into 1.5 ml tube(s).
2. Add a 9–25mm^2 piece of bloodstained material to each tube.
3. Incubate at room temperature for 10 minutes, vortexing every 1–2 minutes.
4. Centrifuge at top speed for 2 minutes and discard the supernatant. Do not disturb the pellet.
5. Place the autoclaved magnetic stir bar in the bottle of ReadyAmp™ Resin and vigorously stir the suspension.
6. While the resin is stirring, remove 200µl aliquots of the resuspended resin to each tube.
7. Vortex the sample(s) to resuspend the pellets.
8. Incubate the sample(s) for 20 minutes in a 56°C water bath or heating block and then vortex for 5–10 seconds.
9. Incubate the sample(s) for 8 minutes in a 100°C water bath or heating block and then vortex for 5–10 seconds.
10. Centrifuge the sample(s) at top speed for 2 minutes. The isolated ssDNA will be in the supernatant. Store at 4°C or –20°C.

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