BIOTECH SUPPORT GROUP

ProPrep™ Genomic 96

96 Filter Well Whole Blood DNA Purification System with ProCipitate™

Direct lysis protocol from 50 µl blood, <15 minutes
PCR suitability down to 1 ng template DNA

Product	Size	Item No.
ProPrep™ Genomic 96	96, 50ul cultures	PPG-96
ProPrep™ Genomic 960	960, 50ul cultures	PPG-960

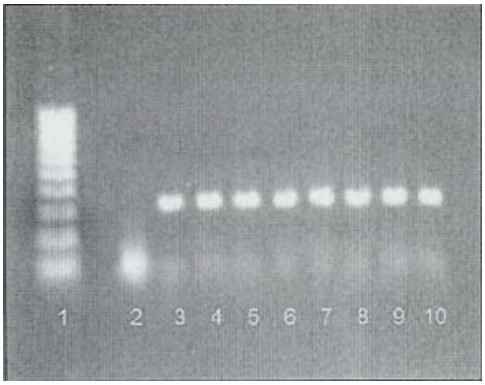
ProPrep™ Genomic 96 is a complete nucleic acid purification system based upon the unique protein extraction reagent, ProCipitate™. The basic protocol includes one step lysis of cells, followed by vacuum removal of contaminating proteins and heme with ProCipitate™.

The ProPrep™ Genomic 96 permits the user to customize a massive PCR or SNP strategy without regard to collecting impractical quantities of whole blood. The isolated DNA is of the highest quality, and PCR can be achieved from as little as 1 ng of template DNA. This means that over 1,000 PCR reactions can be obtained from one, 50 µl whole blood sample.

Centrifugation is optional in the protocol, so it is readily adaptable to automation.

BENEFITS

- High Yield – no bound DNA
- Simple – no specialized equipment
- Minimum Handling – 96 well format
- Safe – non-hazardous solid phase
- Fast – no reduction to buffy coat



Lane 1: 100-1000 base pair Ladder
Lane 2: Negative Control
Lane 3-10: PCR amplicons from 1 ng template DNA purified from whole blood, randomly selected from 96 wells. Amplicons are 280 base pairs from Human HLA-DR-Beta primers at 32 cycles.

MATERIALS AND SCOPE OF SUPPLY

Items Required	ProPrep™ Genomic 96	Storage
Isopropanol (96-100%)	Optional	----
GL1 Lysis Buffer	Supplied	4°C
TR3 Resuspension Buffer	Supplied	Room Temp.
ProCipitate™	Supplied	4°C (best if used before date on label)
96 well filter plate	Supplied	----
96 deep well plate	2 Supplied	----
Plate seal tape	Supplied	----
Wide Bore Pipette Tips	Not Supplied	----

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PROTOCOL - Based on 50 µl of whole blood

1. Add 100 µls of lysis reagent GL1 to each 50 µl blood sample, tape seal the plate and vortex for 30 seconds, incubate 10 minutes at 65°C and vortex again briefly.

2. Shake ProCipitate™ well to completely resuspend. Using a wide bore pipette tip, add 250 µls of ProCipitate™ to each well, mix by pipetting up and down 8-10 times to insure that each sample is homogeneous. IMPORTANT NOTE: Failure to mix thoroughly will result in improper filtration.

3. Incubate for 5 minutes at room temperature.

4. Transfer each sample to the corresponding well of the filter plate and vacuum filter; see tips on next page. The collected supernatant contains the nucleic acids. If necessary after filtration, enzymatic digestion may be performed to remove residual RNA; RNAse not included as part of this kit.

Options – After filtration, the purified DNA is contained within the lysis buffer. The DNA can then be either alcohol precipitated using the "Alcohol Precipitation Protocol", or simply diluted using the "Dilution Protocol", to eliminate inhibitory effects of the lysis buffer.

Alcohol Precipitation Protocol

5. Add 250 µl of isopropanol (room temperature) to each well of collected supernatant.

6. Mix on a shaker for 30 minutes at 100 rpm (room temperature).

7. Centrifuge at 2,000 x g for 10 minutes.

8. Carefully decant supernatant and air dry the pellet at room temperature (≈ 15 min).

9. Resuspend the DNA in 20 - 50 µl of TR3 or other suitable buffer. Incubate at 55°C while shaking at 200 rpm for 30 minutes.

Dilution Protocol

The volume recovered after filtration is approximately 250 µl. A minimum 1:10 dilution is made with DI water. To achieve the maximum number of PCR reactions per sample, dilution up to 1:50 can be made. Typically 10 µl of the diluted purified DNA is utilized as template for PCR.

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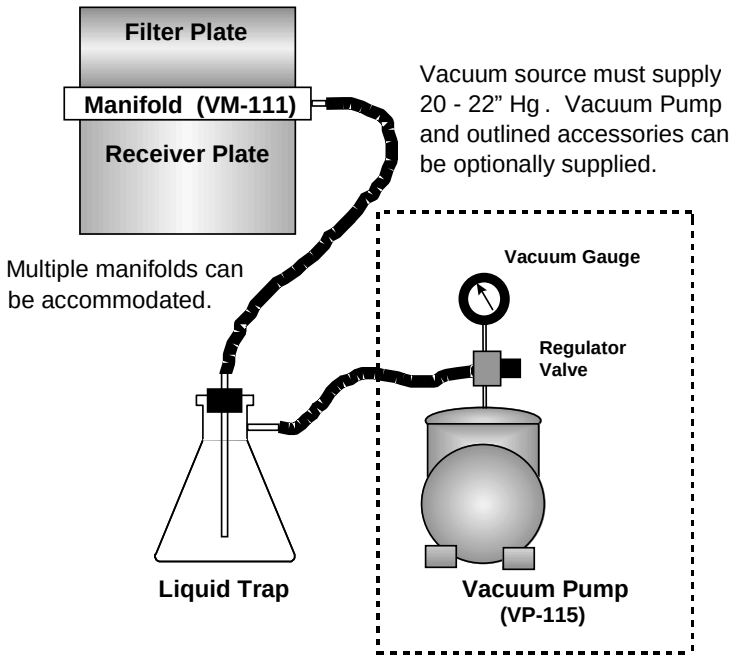
System Layout

The 96 well Filter Plate System eliminates centrifugation for the removal of all suspended solids, quickly and in one step. Filtrate is subsequently ready for alcohol precipitation.



General Filtration Techniques for ProPrep™ Genomic 96

Inspect the gasket faces to insure that they are free of any foreign matter. Prior to adding samples, place filter plate on the vacuum manifold over receiver plate.

Transfer each sample to its corresponding filter well using wide bore pipette tips. Apply full vacuum (≈ 23"Hg) until there is no visible liquid in wells. A cake of solid material will become visible and may crack at the bottom of the wells. This is normal. Continue to apply vacuum for 5 - 15 minutes or until vacuum drops noticeably, greater than 1" Hg.

After filtration is completed and vacuum has been turned off, before removing the plates from the manifold, perform the following: Holding the plates together firmly, raise the assembly approximately 1 inch from the bench top and tap the assembly firmly down onto the bench top. Repeat. This dislodges any remaining supernatant that may remain clinging to the bottom of the plate.

Discard filter plate taking necessary biohazard precautions.

CONTACT US

We welcome your questions and comments regarding our products.

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