BlastR™
Rapid Filtration Kit

contains 50 BlastR™ Filters to rapidly remove genomic DNA from cell lysates

Cat. # BLR02

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Manual Contents

I: Introduction: Overview

Overview

BlastR™ Filters were designed to allow rapid removal of genomic DNA from cell lysates that have become viscous due to nuclear lysis. BlastR™ filters will remove genomic DNA from lysates prepared in denaturing or non-denaturing buffers and are compatible with all commonly used cell lysis buffers, including Laemmli and RIPA buffers.

Lysate viscosity is eliminated after passage over the BlastR™ filter, providing a user friendly lysate for downstream applications including SDS-PAGE, western blotting, immunoprecipitations (IPs) and co-IPs (in cases where a non-denaturing buffer causes cell viscocity due to nuclear lysis).

II: Kit Contents

This kit contains 50 BlastR™ filters (Part # BLR02F). Each filter will remove genomic DNA from up to 1 ml lysate volume per filter. Filters are stable at room temperature for 1 year from purchase. Filters should not be exposed to strong sunlight for prolonged periods.

III: How to use BlastR™ Filters

Method

1) Grow and treat cells as required (a 6 cm plate should yield sufficient lysate for multiple SDS-PAGE samples).

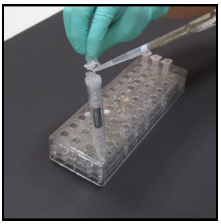
2) Remove culture media and wash the cells once with room temp. PBS.

3) Lyse cells using a buffer of choice. Lysate should become viscous due to release of nuclear genomic DNA.

4) Use a pipette to transfer the crude lysate into a BlastR™ filter (see Fig. 1 A-B).

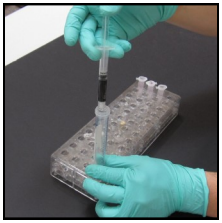
Figure 1: BlastR™ Filter Operation

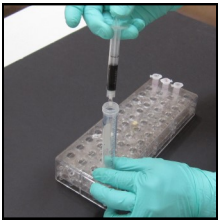
A 

B 

A) BlastR™ filter comprises a syringe barrel that contains the BlastR™ filter and a plunger component.

B) Crude, viscous cell lysate is transferred into the syringe barrel.

C 



C) The plunger is inserted into the syringe barrel and the filter is compressed all the way into the barrel until resistance prevents further compression.

D 

D) Filtered lysate is collected.

5) Use a supplied filter plunger to compress the BlastR™ filter and collect the lysate flow through, including any bubbles that may be eluted at the end of the compression step, into a clean tube (Fig. 1 C-D).

Note: see Appendix 1; Figure 2 for example data from lysates with or without genomic DNA removal using the BlastR™ filter.

6) Centrifuge the lysate at approximately 10,000 g for 1 minute at 4°C.

7) Transfer the lysate to a new tube. The non-viscous lysate is now ready for use in downstream applications.

IV: Troubleshooting

Observation	Possible cause	Remedy
Recovered volume of lysate is <80% of original lysate volume	1) Insufficient pressure applied to plunger	1) The plunger should be pressed slowly and firmly down on the filter to compress the filter and squeeze out >80% of the lysate volume. Lysate bubbles emerging from the syringe is an indicator that you have applied sufficient pressure to elute >80% of the original cell lysate volume.

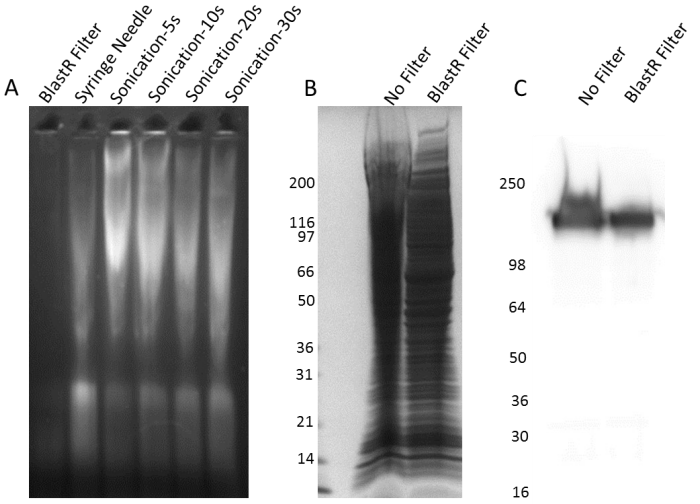


Figure 2. ***BlastR lysis filter is effective at removing genomic DNA.*** (A) A431 cells were lysed with a denaturing lysis buffer. Genomic DNA was removed or sheared with BlastR filter, syringe needle or sonication for 5, 10, 20, and 30 seconds. 2% of lysate was analyzed by ethidium bromide, agarose gel electrophoresis. (B) Lysate from A431 cells lysed with a denaturing buffer was either unfiltered or filter with the BlastR filter. Sample were separated with SDS-PAGE and visualized using Coomassie stain. (C) Duplicate samples from B were separated by SDS-PAGE, transferred to PVDE, and EGFR protein was examined using an EGFR anti-

VI: Changes Made from previous manual version

Changes made from previous method

1) Example data generated from product use has been added to Appendices.

VII: Product Use Information

Limited Use Statement

BlastR™ kits and reagents are based on technology developed at Cytoskeleton Inc. and are the subject of patent applications assigned to Cytoskeleton Inc. The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of product and components of product in research conducted by the buyer. The buyer cannot sell or otherwise transfer this product or any component thereof to a third party or otherwise use this product or its components for commercial purposes. Commercial purposes include, but are not limited to: use of the product or its components in manufacturing; use of the product or its components to provide a service; resale of the product or its components.

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