BlastR™
Rapid Lysate Prep Kit

(suitable for SDS-PAGE or IP Applications*)

50 Assays

Cat. # BLR01

* BlastR™ lysates are not suitable for co-IP assays

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Manual Contents

I: Introduction: Overview

Overview

The BlastR™ Buffer and Filter system allows efficient extraction of proteins from all cellular compartments (Appendix I; Figure 1). The Lysate viscosity is eliminated after passage over the BlastR™ filter (Appendix I, Figure 2), providing a user friendly lysate for downstream applications including SDS-PAGE, western blotting, immunoprecipitations (IPs). BlastR™ lysis buffer is compatible with standard colorimetric protein assays unlike Laemmli and 1% denaturing lysis buffer (Appendix 1; Figure 3).

It should be noted that the BlastR™ Buffer will efficiently break up protein:protein interactions and is therefore not suitable for co-IP applications. The BlastR™ filters are sold separately and can be used with any nuclear lysis buffer system to reduce lysate viscosity by removing genomic DNA from the sample.

II: Kit Contents: Reconstitution and Storage

This kit contains enough reagents for approximately 50 lysate preparations of 500 µl volume of lysis buffer per lysate. Many of the kit components are provided in lyophilized form. Prior to beginning the assay, you will need to reconstitute several components as shown in Table 1. When properly stored and reconstituted, components are guaranteed stable for 6 months.

Table 1: Kit Contents and storage prior to reconstitution of components

Kit Component	Cat. # /Part # (Quantity)	Reconstitution	Storage after reconstitution
BlastR™ Lysis Buffer	Part# BLST01 2 bottles	Resuspend in 15 mls sterile water to give a 1X stock solution	4°C
BlastR™ Dilution Buffer	Part# BDB01 1 bottle	Resuspend in 130 mls sterile water to give a 1X stock solution.	4°C
Protease Inhibitor Cocktail	Cat# PIC02 1 tube	Resuspend in 1 ml of DMSO (provided in kit) for a 100X stock solution	-20°C
Precision Red™ Advanced Protein Assay Reagent	Part #GL50 1 bottle (100 ml)	Not required	Room temp.
DMSO	Part# DMSO 1 tube (1.5 ml)	Not required	Room temp.
5X Laemmli Buffer (SDS sample buffer)	Part# SDS01 1 tube	Not required	Room temp.
BlastR™ filters	Cat# BLR02 50 filters	Not required	Room temp.

* Items with part numbers (Part #) are not sold separately and available only in kit format. Items with catalog numbers (Cat. #) are available separately.

Standard reagents and equipment that you will require but are not supplied:

- Tissue culture cells or tissue of interest
- PBS pH 7.4 buffer (10 mM phosphate buffer pH 7.4, 140 mM NaCl, 3 mM KCl)
- Cell scrapers
- Lysate specific inhibitors
- Liquid nitrogen for snap freezing cell lysates. Recommended if lysates will be used for immunoprecipitations (IPs).

III: Lysate Preparation for SDS-PAGE Applications

For SDS-PAGE based applications, such as protein visualization, e.g. coomassie staining or western blot analysis, cell lysates do not need to be diluted in BlastR™ Dilution Buffer and can be used directly as BlastR™ Lysis Buffer lysates. See Section IV for lysate preparations that will be used in affinity purification or immunoprecipitation applications.

SDS-PAGE Application Method Sample Preparation: Cell Culture

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 twice with 1X PBS.
3. Add 500 µl of BlastR™ lysis buffer (supplemented with 5 µl of 100X protease inhibitor cocktail, and other necessary inhibitors) and lyse cells using a cell scraper. The lysate will become viscous due to nuclear lysis.
4. Use a snipped 1 ml pipette to transfer the crude lysate into a BlastR™ filter that has been placed in a 15 ml collection tube (see Appendix II).
5. Use a supplied filter plunger to completely compress the BlastR™ filter and collect the lysate flow through, including any bubbles , in a 15 ml tube (see Appendix II).
6. Optional: transfer to a 1.5 ml microcentrifuge tube and centrifuge the lysate at approximately 10,000 g for 1 minute at 4°C. Transfer to a new microcentrifuge tube.
7. Quantitate protein concentration (see SDS-PAGE quantitation below).

SDS-PAGE Application Method Sample Preparation: Tissue

Table 2: Example of Total Protein Obtained From 100 mg of Tissue

Tissue Type	Tissue amount (mg)	Total Protein (mg)
Liver	100mg	10-15
Heart	100mg	10-20
Brain	100mg	8-12

Processing Tissue Lysate:

1. Obtain fresh or frozen tissue.
2. Prepare BlastR™ lysis and dilution buffers with protease inhibitor cocktail (100x) and other necessary inhibitors.
3. Add 500 µl of BlastR™ lysis buffer per 50 mg of tissue in an appropriate-sized, glass dounce tissue grinder/homogenizer, and apply 10-12 strokes. The lysate will become viscous due to nuclear lysis.

   Note: The amount of BlastR™ lysis buffer will change depending on the amount of tissue used. For example, if you use 100 mg of tissue then lyse with 1 ml of BlastR™ lysis buffer.
4. Use a snipped 1 ml pipette to transfer the crude lysate into a BlastR™ filter that has been placed in a 15 ml collection tube (see Appendix II).
5. Use a supplied filter plunger to completely compress the BlastR™ filter and collect the lysate flow through, including any bubbles , in a 15 ml tube (see Appendix II).
6. Add 500 µl of filtered lysate with 500 µl of BlastR™ Dilution Buffer in a 1.5 ml microcentrifuge tube and mix.

7. Centrifuge the lysate at approximately 10,000 g for 10 minute at 4°C in a table-top microcentrifuge. Transfer supernatant to a new 1.5 ml microcentrifuge tube.
8. Quantitate protein concentration (see SDS-PAGE quantitation below).

Protein Quantitation Assay for SDS-PAGE Lysate

1) Aliquot 20 µl of cell lysate into a microfuge tube.
2) Dilute lysate with 80 µl of BlastR™ Dilution Buffer and mix well.
3) Make a Blanking Buffer by adding 20 µl of BlastR™ Lysis Buffer to 80 µl of BlastR™ Dilution Buffer.
4) Aliquot 1 ml of Precision Red™ Advanced Protein Assay Reagent into each of two 1ml cuvettes.
5) Add 20 µl of diluted cell lysate to one cuvette and 20 µl of the Blanking Buffer to the second cuvette.
6) Cap the cuvettes and mix well by inverting several times, and incubate for 1 minute at room temperature.
7) Blank the spectrophotometer at 600 nm wavelength with buffer sample from step 3.
8) Read the lysate sample at 600 nm.
9) Use the equation below to determine the lysate protein concentration.

sample reading OD_{600} x 25 = protein concentration in mg/ml

NOTE: *readings below 0.05 or above 0.5 are close to the linear range capacity of the protein assay. For readings >0.5 samples can be diluted. For readings <0.05 more lysate can be added to the ADV02 (up to 50 µl). See Table 3 for multipliers to convert spectrophotometer readings to mg/ml lysate protein concentration.*

10) Equalize lysate protein concentrations using BlastR™ Lysis Buffer.
11) If lysates are to be used for SDS-PAGE one fifth volume of 5X Laemmli buffer (provided in kit) should be addd to the lysate.
12) Lysates that will not be used straight away can be aliquoted and snap frozen in liquid nitrogen and stored at –20 to -80°C. Lysates should be stable for several months. Always aliquot into experiment sized volumes to prevent freeze/thaws.

Table 3: Multiplier values for protein concentration measurements of lysates used for SDS-PAGE Applications

Volume of diluted cell lysate added to 1 ml of Precision Red Protein Assay reagent (µl)	Multiplier to use with sample reading OD_{600}
10	50
20	25
30	16.5
40	12.5
50	10

IV: Lysate Preparation for IP Applications

Applications such as immunoprecipitation (IP) requires dilution of BlastR™ Lysate to preserve affinity reagent integrity. Initial cell lysis in BlastR™ Lysis buffer ensures that proteins are extracted efficiently from all cellular compartments and that protein:protein interactions are disrupted. After cell lysis a 1 in 5 dilution of the BlastR™ Lysis Buffer cell lysate with BlastR™ Dilution Buffer results in a final IP binding buffer that is compatible with IP applications. NOTE: due to disruption of protein:protein interactions, BlastR™ lysates are not suitable for co-IP assays. The following method provides general guidelines for achieving a lysate that is suitable for IP applications.

Immunoprecipitation Application Method Sample Preparation: Cell Culture

It is recommended to aim for 1.0 mg of total protein lysate per IP assay as an optimal starting point. We recommend utilizating150 cm^2 plates.

Protein yield varies widely for any given cell line, and it is strongly recommended to perform a "test plate" protein quantitation (see appendix III).

Processing Tissue Culture Cell Lysate:

1. Grow and treat tissue culture cells as required.
2. Prepare BlastR™ lysis and dilution buffers with protease inhibitor cocktail (100x) and other necessary inhibitors.
3. Remove culture media and wash the cells twice with 10 ml of 1x PBS. Note: remove as much PBS as possible prior to adding BlastR™ lysis buffer in order to maximize cell lysis.
4. Add X µl of BlastR™ lysis buffer (based on expected protein yield; see table 4) and lyse cells using a cell scraper. The lysate will become viscous due to nuclear lysis.

Table 4: BlastR™ Lysis/Dilution Buffer Chart

Plate Protein content	Recommended BlastR™ Lysis Buffer volume	Recommended BlastR™ Dilution Buffer volume
< 1 mg	Combine protein from multiple plates: See appendix VIII, step 9	To make 1.5 ml final volume
1-2 mg	300 µl	To make 1.5 ml final volume
2-4 mg	600 µl	To make 3 ml final volume
4-6 mg	900 µl	To make 4.5 ml final volume

5. Use a snipped 1 ml pipette to transfer the crude lysate into a BlastR™ filter that has been placed in a 15 ml collection tube (see Appendix VII).
6. Use a supplied filter plunger to completely compress the BlastR™ filter and collect the lysate flow through, including any bubbles , in a 15 ml tube (see Appendix VII).
7. Optional: Centrifuge the lysate at approximately 10,000 g for 1 minute at 4°C. Transfer to a new tube.
8. Dilute the lysate with BlastR™ Dilution Buffer to give the final volume given in Table 4. This step is important as the final buffer composition will affect the IP reaction stringency.
9. Quantitate protein concentration (see IP lysate quantitation below).

Immunoprecipitation Application Method Sample Preparation: Tissue

It is recommended to aim for 1.0 mg of total protein lysate per IP assay as an optimal starting point. Table 2 provides examples of protein yield from 100 mg of various tissue types.

Processing Tissue Lysate:

1. Obtain fresh or frozen tissue.
2. Prepare BlastR™ lysis and dilution buffers with protease inhibitor cocktail (100x) and other necessary inhibitors.
3. Add 1 ml of BlastR™ lysis buffer per 100 mg of tissue in an appropriate-sized, glass dounce tissue grinder/homogenizer, and apply 10-12 strokes. The lysate will become viscous due to nuclear lysis.

   Note: The amount of BlastR™ lysis buffer will change depending on the amount of tissue used. For example, if you use 50 mg of tissue then lyse with 500 µl of BlastR™ lysis buffer.
5. Use a snipped 1 ml pipette to transfer the crude lysate into a BlastR™ filter that has been placed in a 15 ml collection tube (see Appendix VII).
6. Use a supplied filter plunger to completely compress the BlastR™ filter and collect the lysate flow through, including any bubbles , in a 15 ml tube (see Appendix VII).
7. Add 200 µl of filtered lysate to a 1.5 ml tube. Snap freeze the remaining lysate.

   Note: Only a fraction of lysate is processed further as 100mg of tissue will produce excess lysate. However, this is tissue type dependent.
8. Dilute the 200 µl of filtered lysate with 200 µl of BlastR™ Dilution Buffer and mix.
9. Centrifuge the lysate at approximately 10,000 g for 10 minute at 4°C in a table-top microcentrifuge. Transfer supernatant to a new 15 ml tube.
10. Add 600 µl of BlastR™ Dilution Buffer to dilute the clarified lysate to a 1 ml final volume. This step is important as the final buffer composition will affect the IP reaction stringency.
11. Quantitate protein concentration (see IP lysate quantitation below).

Protein Quantitation Assay For Immunoprecipitation Lysate

1. Add 1 ml of Precision Red™ Advanced Protein Assay Reagent (Part # GL50) to each of two 1ml cuvettes.
2. Mix 10 µl of BlastR™ Lysis Buffer and 40 µl of BlastR™ Dilution Buffer to a clean tube on ice. This will be used for the protein reading blank sample.
3. Add 20 µl of the Lysis/Dilution buffer mix (from step 2) to the first cuvette and mix by inverting two to three times.
4. Add 20 µl of the diluted cell lysate (from experiment) to the second cuvette, mix as above.
5. Incubate samples for 1 min at room temperature.
6. Blank spectrophotometer with the Lysis/Dilution buffer mix sample (from step 3).

7. Measure absorbance of the lysate sample (from step 4) at 600 nm.
8. Determine the lysate protein concentration as follows;

sample reading OD_{600} x 5 = protein concentration in mg/ml

NOTE: *readings below 0.05 or above 0.5 are close to the linear range capacity of the protein assay. For readings >0.5 samples can be diluted. For readings <0.05 more lysate can be added to the ADV02 (up to 50 µl). See Table 5 below for multipliers to convert spectrophotometer readings to mg/ml lysate protein concentration.*

9. If there is insufficient protein in one plate, it is recommended to use 2 or more plates per IP. In this case plates will be harvested in series, transferring the original 300 µl of Lysis Buffer between plates.
10. Based on protein concentration, dilute sample with a buffer mix (1 part BlastR™ lysis: 4 parts BlastR™ dilution) to a desired final concentration (usually 1 mg/ml).
11. Snap freeze aliquots of any samples that won't immediately be used. Proceed to immunoprecipitation.

Table 5: Multipliers to Convert Spectrophotometer Readings to mg/ml Lysate

Note: Protein concentration readout (mg/mL) will be lower due to dilution of lysate with dilution buffer prior to measuring the protein concentration. However, this is factored into the multiplier.

Volume of cell lysate added to 1 ml of Precision Red Protein Assay reagent (µl)	Multiplier to use with sample reading OD_{600}
10	10
20	5
30	3.3
40	2.5
50	2.0

V: 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.

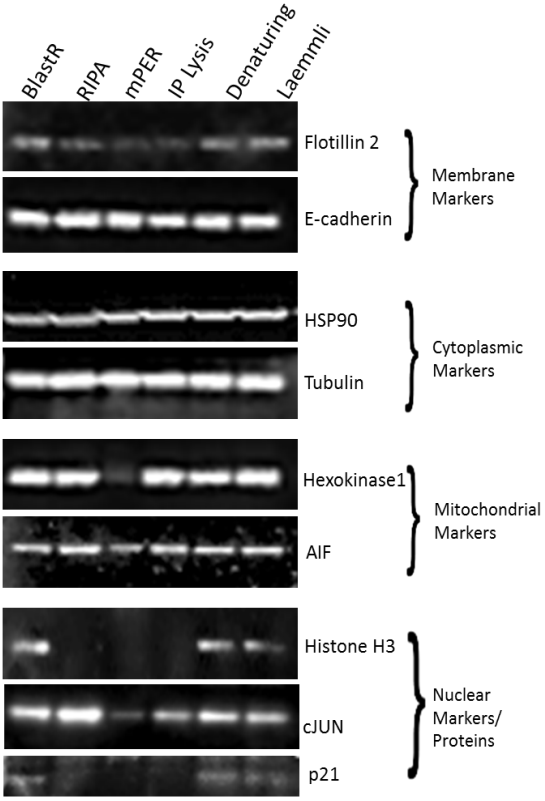


Figure 1. ***Comparison of BlastR lysis buffer to alternative lysis buffers.*** A431 cells were lysed with BlastR, RIPA, mPER, IP lysis, Denaturing (1% SDS), and Laemmli lysis buffers. All denaturing lysates had genomic DNA removed using BlastR filter. Isolation of proteins from the membrane, cytoplasmic, mitochondrial, and nuclear markers were determined using antibodies against the respective compartment marker proteins.

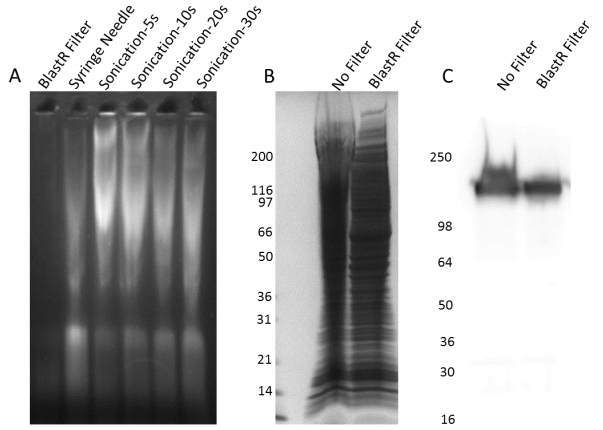


Figure 2. ***BlastR lysis filter is effective at removing genomic DNA.*** (A) A431 cells were lysed with a denaturing lysis buffer. Genomic DNA was removed or sheared with BlastR filter, syringe needle or sonication for 5, 10, 20, and 30 seconds. 2% of lysate was analyzed by ethidium bromide, agarose 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-

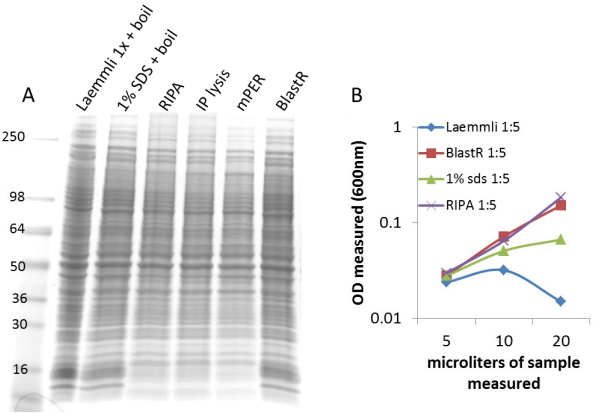
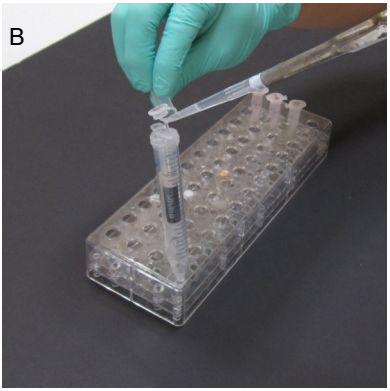
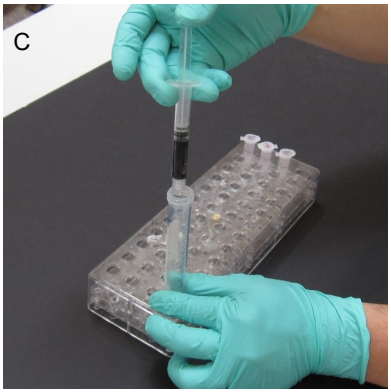


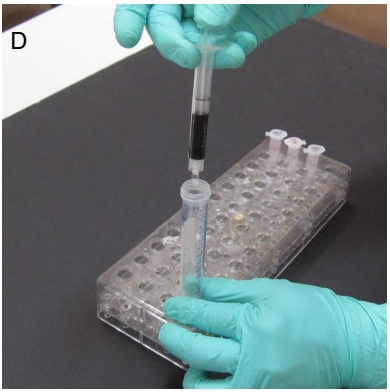
Figure 3. ***BlastR lysis buffer characteristics.*** (A) A431 cells were lysed with BlastR, RIPA, mPER, IP lysis, Denaturing (1% SDS), and Laemmli lysis buffers. All denaturing lysates had genomic DNA removed using the BlastR filter. Coomassie stain was performed to obtain a protein isolation profile with these buffers. (B) Protein quantitation of RIPA, BlastR, 1% SDS, and Laemmli was performed using a standard colorimetric assay (ADV02). A titration of 5, 10, and 20 μL was performed to determine the

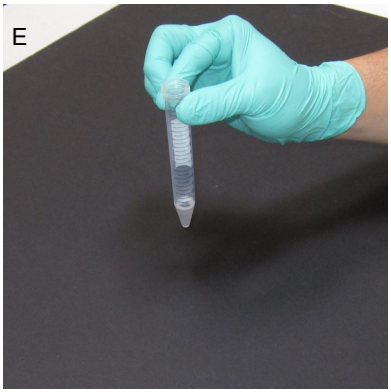
Figure 4: Filtering genomic DNA from cell lysate

Cytoskeleton's proprietary filtering system provides end-users with a very fast and simple method to remove genomic DNA from viscous cell lysates. This page shows example pictures that depicts utilization of the filter system.









A: BlastR™ Lysate Filter

B: Lysate is loaded into the filter that was placed in a15ml tube

C: Plunger is placed into the syringe and lysate is passed through the filter by compression

D: Collect lysate, including bubbles through complete compression

E: Filtered lysate

Appendix III: Test Plate for IP applications

Test Plate to Determine Protein Concentration

It is recommended to aim for 1.0 mg of total protein lysate per IP assay as an optimal starting point.

Protein yield varies widely for any given cell line, and it is strongly recommended to perform a "test plate" protein quantitation, particularly if you are unsure of the expected protein yield from your experimental conditions. This is a simple procedure and is performed as follows;

Cell lysate preparation with BlastR™ Lysis System

1. Grow and treat cells as required (The volumes used in this protocol assume the use of a 150 cm^2 plate).
2. Remove culture media and wash the cells twice with 10 ml of room temp PBS. Note: remove as much PBS as possible prior to adding BlastR™ lysis buffer in order to maximize cell lysis.
3. Add 300 µl of BlastR™ lysis buffer (supplemented with 3 µl of 100X protease inhibitor cocktail) and lyse cells using a cell scraper. The lysate will become highly viscous due to nuclear lysis.
4. Use a 1 ml pipette to transfer the crude lysate into a BlastR™ filter that is in a 15 ml collection tube (see Appendix II). Note: using a cut pipette tip may aide in transferring the viscous lysate.
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 (see Appendix II).
6. Optional: Centrifuge the lysate at approximately 10,000 g for 1 minute at 4°C. Transfer to a new tube and quantitate the lysate protein concentration as described below.

Protein Quantitation for BlastR™ Cell Lysate

1. Aliquot 20 µl of cell lysate into a microfuge tube.
2. Dilute lysate with 80 µl of BlastR™ Dilution Buffer and mix well.
3. Make a Blanking Buffer by adding 20 µl of BlastR™ Lysis Buffer to 80 µl of BlastR™ Dilution Buffer.
4. Aliquot 1 ml of Precision Red™ Advanced Protein Assay Reagent into each of two 1ml cuvettes.
5. Add 20 µl of diluted cell lysate to one cuvette and 20 µl of the Blanking Buffer to the second cuvette.
6. Cap the cuvettes and mix well by inverting several times.
7. Incubate the samples for 1 minute at room temperature.

1. Blank the spectrophotometer against the Blanking Buffer sample at 600 nm wavelength.
2. Read the lysate sample at 600 nm.
3. Use the equation below to determine the lysate protein concentration.

sample reading OD_{600} x 25 = protein concentration in mg/ml

NOTE: *readings below 0.05 or above 0.5 are close to the linear range capacity of the protein assay. For readings >0.5 samples can be diluted. For readings <0.05 more lysate can be added to the ADV02 (up to 50 µl). See Table 6 for multipliers to convert spectrophotometer readings to mg/ml lysate protein concentration.*

Table 6: Multiplier values for protein concentration measurements of lysates

Volume of diluted cell lysate added to 1 ml of Precision Red Protein Assay reagent (µl)	Multiplier to use with sample reading OD_{600}
10	50
20	25
30	16.5
40	12.5
50	10

Calculating Total Protein Concentration Per Plate

1. Multiply protein concentration (mg/ml) by 0.3 ml (protein volume) to determine test plate protein content.

   Protein concentration x volume = Test plate protein content (mg)

2. If the test plate protein content is > 2 mg then a higher volume of lysis buffer should be used (see Table 4). NOTE: it is important to maintain the recommended ratio between Lysis and Dilution buffers as the final buffer composition of the lysate will influence the stringency of PTM enrichment.
3. If there is insufficient protein in one plate, it is recommended to use 2 or more plates per IP. In this case plates will be harvested in series, transferring the original 300 µl of Lysis Buffer between plates.
4. When performing the Assay Protocol in section IV, the protein concentration readout (mg/mL) will be lower due to dilution of lysate with dilution buffer prior to measuring the protein concentration; however, total protein per plate will remain the same.

VI: Changes Made from previous manual version

Changes made from previous versions

1) Addditional information regarding product performance has been added to appendices.

VII: Limited Use Statement

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.

The terms of this Limited Use Statement apply to all buyers including academic and for-profit entities. If the purchaser is not willing to accept the conditions of this Limited Use Statement, Cytoskeleton Inc. is willing to accept return of the unused product with a full refund.

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