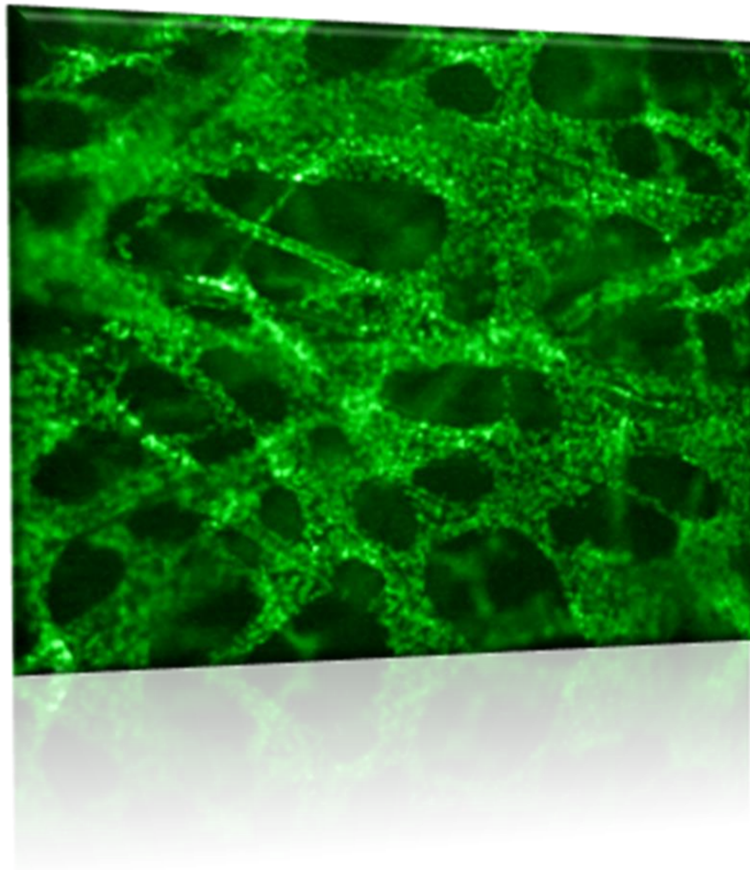
SEEDEZ™ PROTOCOLS

EXTRACTION AND QUANTIFICATION OF TOTAL PROTEIN ISOLATED FROM 3D CELL CULTURES IN



THE SEEDEZ™

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Extraction and Quantification of Total Protein Isolated From 3D Cell Cultures in the SeedEZ™

December 2018

TABLE OF CONTENTS

INTRODUCTION ______ 1

PROTEIN EXTRACTION FROM CELLS CULTURED IN THE SeedEZ™ ______ 1

PROTEIN QUANTIFICATION ASSAY ______ 2

BCA protein assay principle ______ 2

Results: 4-Day brain cell cultures in uncoated and PDL coated SeedEZ™ substrates ______ 2

Results: 7-Day brain cell cultures in gelled Matrigel in the SeedEZ™ substrates ______ 4

INTRODUCTION

Protein extraction is used to isolate proteins from cells and tissues for downstream analysis; for example, for protein quantification, separation, labeling, visualization, purification and further detection. A protein assay is used to quantify the amount of protein isolated from cells before chromatographic, electrophoretic, immunochemical and other studies. The below protocols show how to extract total protein from cells cultured in 3D in the SeedEZ™ under different culturing conditions and how to apply a protein assay to quantify it. However, you may extract and quantify total protein by following protocols your normally use.

PROTEIN EXTRACTION FROM CELLS CULTURED IN THE SeedEZ™

This protocol may be used with Thermo Scientific Pierce® BCA Total Protein Assay. If a different protein assays if used, please follow manufacturer guidelines for protein extraction including recommendations for use of specific detergents and protease inhibitors.

This protocol is for three-dimensional (3D) cell cultures cultured in the SeedEZ™ SC-C048 substrates. Recommended volumes are for this substrate only. The same protocol may be used for 3D cell cultures embedded in uncoated or coated SeedEZ™ substrates and 3D cell cultures embedded in a hydrogel in the SeedEZ™.

EXTRACTION BUFFER

- 0.5% NP-40
- 0.5% Deoxycholate (deoxycholic acid)
- 150 mM NaCl
- 50 mM Tris
- Adjust pH to 7.4 with HCl

METHODS

1. Aspirate medium from cultures in the SeedEZ™ SC-C048.
2. Rinse 4X with cold PBS.
3. Add 60 µl of extraction buffer to each SeedEZ™ substrate.
4. Transfer the plate to shaker and shake for 1 hour on ice.
5. Collect buffer from each well/substrate and transfer into a respective conical tube.
6. Spin at 1000 x g for 5 minutes at 4°C.
7. Transfer supernatant to a fresh tube.
8. Perform protein assay (2 x 25 µl samples for each replicate per condition) or transfer to -80 ^{o}C for later use.

PROTEIN QUANTIFICATION ASSAY

Protein concentrations were quantified using Pierce® BCA Total Protein Assay Kit #23227 relative to BSA standards.

BCA PROTEIN ASSAY PRINCIPLE

The Thermo Scientific Pierce BCA Protein Assay is a detergent-compatible formulation based on bicinchoninic acid (BCA) for the colorimetric detection and quantification of total protein. This method combines the well-known reduction of Cu^{+2} to $Cu^{+}1$ by protein in an alkaline medium (the biuret reaction) with the highly sensitive and selective colorimetric detection of the cuprous cation (Cu^{+1}) using a unique reagent containing bicinchoninic acid. The purple-colored reaction product of this assay is formed by the chelation of two molecules of BCA with one cuprous ion. This water-soluble complex exhibits a strong absorbance at 562 nm that is nearly linear with increasing protein concentrations over a broad working range (20-2,000 µg/ml). The BCA method is not a true end-point method; that is, the final color continues to develop. However, following incubation, the rate of continued color development is sufficiently slow to allow large numbers of samples to be assayed together. The macromolecular structure of protein, the number of peptide bonds and the presence of four particular amino acids (cysteine, cystine, tryptophan and tyrosine) are reported to be responsible for color formation with BCA. Studies with di-, tri- and tetrapeptides suggest that the extent of color formation caused by more than the mere sum of individual color producing functional groups. Accordingly, protein concentrations generally are determined and reported with reference to standards of a common protein such as bovine serum albumin (BSA). A series of dilutions of known concentration are prepared from the protein and assayed alongside the unknown(s) before the concentration of each unknown is determined based on the standard curve.

METHODS

1. Prepare diluted Albumin (BSA) standards according to Pierce® BCA Protein Assay Kit instructions.
2. Prepare BCA Working Reagent (WR) according to Pierce® BCA Protein Assay Kit instructions.
3. Follow Microplate Procedure, Sample to Working Reagent 1:8 in Pierce® BCA Protein Assay Kit instructions for each standard or unknown sample replicate.
4. Measure absorbance at or near 562 nm on a plate reader.
5. Generate standard curve and quantify total protein relative to BSA standards.

RESULTS: 4-DAY BRAIN CELL CULTURES IN UNCOATED AND PDL COATED SEEDEZ™ SUBSTRATES

Mixed 3D cell cultures of brain cells were seeded into uncoated and Poly-D-Lysine coated SeedEZ™ SC-C048 substrates placed in wells of a 48-well plate. The mixed population of cells comprised primary cortical neurons and secondary (1X passaged) astrocytes and microglia. At seeding, the cell ratio was 2:1 neuron:glia at a total live cell density of 4 x 10^6 cells/ml. Cells were seeded in 60 µl medium suspension into the substrates. At seeding there were 240,000 cells per substrate.

Coated substrates were coated overnight in 100 µg/ml Poly-D-Lysine coating solution in sterile DI water in a cell culture incubator and then rinsed 2X with sterile DI water. The last rinse was aspirated from the substrates prior to cell seeding.

Cells were cultured for 4 days. During first 3 days in culture the medium composition was Neurobasal + 2% B-27(-AO) + 1% G-5 + 0.5 mM GlutaMAX. After 3 days in culture, the medium was replaced with Neurobasal + 2% B-27(-AO) + 0.5 mM GlutaMAX and cells cultured for one more day, after which Thermo Scientific Pierce® BCA Total Protein Assay was done.

For cell maintenance prior to seeding, Poly-D-Lysine coating, cell dissociation and cell seeding, please see the SeedEZ™ Cell Seeding Protocols.

The following 3D culturing conditions and suitable controls were setup and protein quantified relative to BSA standards:

Label	Culturing conditions and respective controls	Number of replicates	Protein (μg/ml ±SD) per substrate
-PDL / -Cells	Uncoated SeedEZ™ in an uncoated well	4	115 ± 75
-PDL / +Cells	3D cell culture in an uncoated SeedEZ™	4	260 ± 106
+PDL / -Cells	Poly-D-Lysine coated SeedEZ™ in a Poly-D-Lysine coated well	4	224 ± 106
+PDL / +Cells	3D culture in a PDL-coated SeedEZ™ seated in a PDL-coated well	4	565 ± 30

Since uncoated SeedEZ™ is not cell adhesive enough for these cell types, it was expected that the total number of cells in uncoated SeedEZ™ substrates would be lower than in coated SeedEZ™ substrates. Results

SeedEZ - Pierce BCA Protein Assay

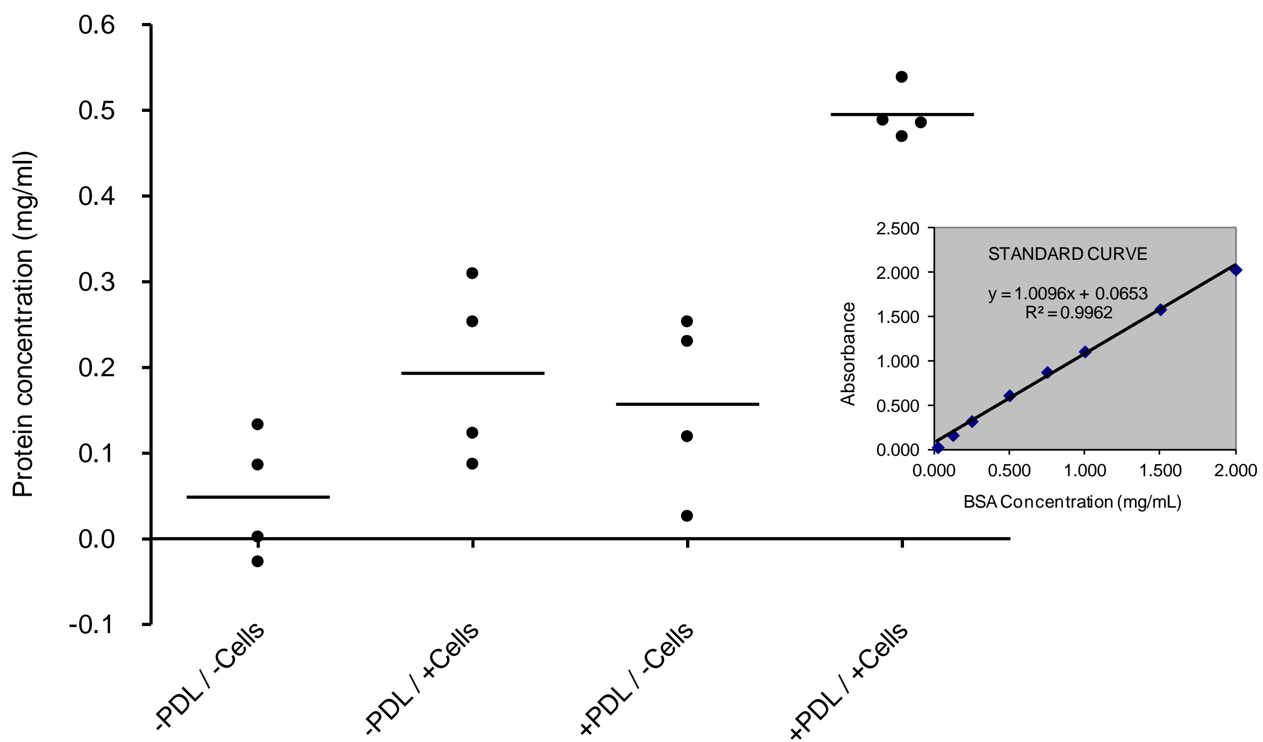

Fig. 1 Total protein from 3D cell cultures cultured in uncoated and PDL-coated SeedEZ for 4 days

are shown in Fig. 1.

RESULTS SHOW THE FOLLOWING:

I. Cell protein can be extracted from 3D cell cultures embedded in the SeedEZ™.

II. Cell protein can be quantified using a standard protein assay with respect to suitable controls.

III. Poly-D-Lysine coating is effective in confining brain cells to the interior of the SeedEZ™.

RECOMMENDATIONS

When using coated or uncoated SeedEZ™ substrates, setup suitable controls.

Before adding extraction buffer, you may rinse SeedEZ™ substrates more than 4X to remove any remaining Albumin from medium or other constituents used during culturing known to interfere with the assay; for example, Phenol red.

RESULTS: 7-DAY BRAIN CELL CULTURES IN GELLED MATRIGEL IN THE SEEDEZ™ SUBSTRATES

Mixed 3D cell cultures of brain cells were seeded in 7.5 mg/ml protein Growth Factor Reduced Matrigel ice-cold sol-state suspension into uncoated SeedEZ™ SC-C048 substrates placed in wells of a 48-well plate. The mixed population of cells comprised primary cortical neurons and secondary (1X passaged) astrocytes and microglia. At seeding, the cell ratio was 2:1 neuron:glia at a total live cell density of 4 x 10^6 cells/ml. Cells were seeded in 60 µl medium suspension into the substrates. At seeding there were 240,000 cells per substrate.

Cells were cultured for 7 days. During first 3 days in culture the medium composition was Neurobasal + 2% B-27(-AO) + 1% G-5 + 0.5 mM GlutaMAX. After 3 days in culture, the medium was replaced with Neurobasal + 2% B-27(-AO) + 0.5 mM GlutaMAX and cells cultured for another 4 days, after which Thermo Scientific Pierce® BCA Total Protein Assay was done.

For cell maintenance, cell dissociation and cell seeding in Matrigel, please see the SeedEZ™ Cell Seeding Protocols.

The following 3D culturing conditions and suitable controls were setup and protein quantified relative to BSA standards:

Label	Culturing conditions and respective controls	Number of replicates	Protein (mg/ml ±SD) per substrate
7.5 mg/ml Matrigel	SeedEZ™ comprising gelled Matrigel	5	1.33 ± 0.12
7.5 mg/ml Matrigel + Cells	3D cell culture in Matrigel in the SeedEZ™	3	1.56 ± 0.06

Results are shown in Fig. 2.

RESULTS SHOW THE FOLLOWING:

I. Cell protein can be extracted from 3D cell cultures embedded and cultured in a gelled 7.5 mg/ml extracellular matrix protein concentration Matrigel in the SeedEZ™.

II. Cell protein can be quantified using a standard protein assay using suitable controls.

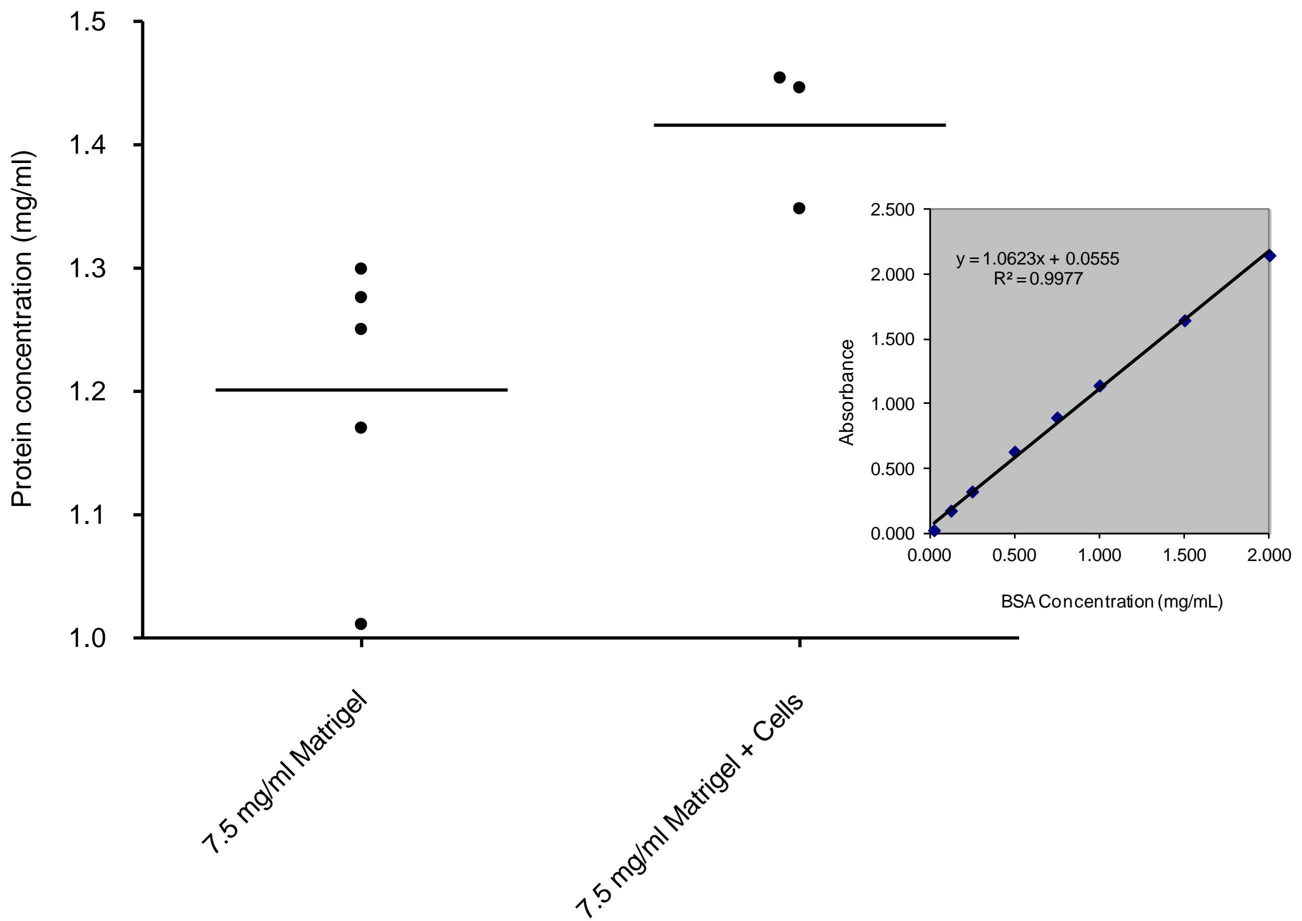

Fig. 2 Total protein from 3D cell cultures cultured in gelled 7.5 mg/ml Matrigel in the SeedEZ for 7 days

RECOMMENDATIONS

When using extracellular matrices or other hydrogels embedded in the SeedEZ™ during culturing, setup suitable controls.

Before adding extraction buffer, you may rinse SeedEZ™ substrates more than 4X to remove any remaining Albumin from medium "trapped" in hydrogel in the SeedEZ™ or other constituents used during culturing known to interfere with the assay; for example, Phenol red.

Depending on extraction buffer used, an amount of protein from the extracellular matrix gel (in addition to cell protein) may be extracted. For all these reasons, setup suitable controls.