Bio-Plex phosphoprotein assay using spinal cords and skeletal muscles of mice

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Method Article

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Abstract

Bio-Plex phosphoprotein assays are magnetic bead-based immunoassays for the detection of intracellular phosphoproteins in cell and tissue lysates. The assay can be readily multiplexed to simultaneously examine the expression levels of numerous phosphorylated proteins.

Introduction

This protocol was used in our *Nature Communications* paper.

Reagents

Bio-Plex Pro™ Cell Signaling Assay kit (Bio-Rad Laboratories)

DC™ Protein Assay

Equipment

Tissue grinder

Scissors

Freezer

Sonicater

Aluminum foil

Eppendorf Tubes Centrifuge

Plate reader for measuring protein concentration

Magnetic washer

Plate shaker

Luminex® 200 xPONET® 3.1 system (Merck Millipore)

Procedure

Tissue preparation

1. Cut the tissue into small pieces (~3x3 mm) in a 1.5 ml tissue grinder
2. Add 10 times volume of cell lysis buffer and grind the tissue on ice using 20 strokes

3. Freeze samples at -80℃. Freezing and thawing samples helps increase cell lysis effects.

4. Thaw the sample and sonicate on ice (1 min)

5. Centrifuge at 15000xg for 10 min at 4℃

6. Collect the supernatant

7. Measure the protein concentration using DC™ Protein Assay and a plate reader

8. Adjust the concentration of protein to 600µg/ml with cell lysis buffer

9. Freeze samples at -80℃

Assay day1

1. Thaw sample lysates and keep on ice

2. Reconstitute lyophilized cell lysate control with 250 µl of dH_2O, vortex for 5 sec to mix and incubate at room temperature for 20 min

3. Centrifuge all samples and lysate controls at 15000xg for 10 min at 4℃

4. Vortex the 20x coupled beads at mid speed for 30 sec and carefully open the cap

5. Dilute coupled beads to 1x with wash buffer, vortex, protect from light with aluminum foil. For the assay in 96wells, mix 288 µl of 20x beads with 5472 µl of wash buffer.

6. Vortex the 1xbeads for 15 sec at medium speed and transfer 50 µl to each well of the assay plate

7. Wash the plate two times with 200 µl wash buffer using magnetic washer

8. Transfer 50 µl of each sample and Bio-Rad cell lysate control to the appropriate wells of the plate. Use detection antibody diluent as the blank.

9. Cover the plate with a new sheet of sealing tape and incubate in the dark for 15-18 hr at room temperature with shaking. Shake at 900-1100 rpm for 30 sec and turn down to 300-450 rpm.

Assay day2

1. Start up, warm up, and calibrate Luminex® 200 xPONET® 3.1 system

2. Bring all buffers and diluents to room temperature

3. Vortex the 20x detection antibodies for 15-20 sec at medium speed

4. Dilute detection antibodies to 1x with detection antibody diluent. For 96wells, mix 150 µl of 20x detection antibodies with 2850 µl of detection antibody diluent.

5. Slowly remove and discard the sealing tape of the assay plate

6. Wash the plate three times with 200 µl wash buffer using magnetic washer

7. Vortex the 1x detection antibodies and transfer 25 µl to each well of the assay plate

8. Cover the plate with a new sheet of sealing tape and incubate in the dark for 30 min at room temperature with shaking. Shake at 900-1100 rpm for 30 sec and turn down to 300-450 rpm.

9. Vortex the 100x Sreptavidin-PE (SA-PE) for 5 sec at medium speed

10. Dilute SA-PE to 1x with detection antibody diluent. For the assay in 96wells, mix 150 µl of 20x detection antibodies with 2850 µl of detection antibody diluent.

11. Slowly remove and discard the sealing tape of the assay plate

12. Wash the plate three times with 200 µl wash buffer using magnetic washer

13. Vortex the 1x SA-PE and transfer 50 µl to each well of the assay plate

14. Cover the plate with a new sheet of sealing tape and incubate in the dark for 10 min at room temperature with shaking. Shake at 900-1100 rpm for 30 sec and turn down to 300-450 rpm.

15. Slowly remove and discard the sealing tape of the assay plate

16. Wash the plate three times with 200 µl wash buffer using magnetic washer

17. To resuspend beads for plate reading, add 125 µl resuspension buffer to each well. Cover the plate with a new sheet of sealing tape and shake at 900-1100 rpm for 30 sec.

18. Slowly remove the sealing tape and place the plate on the reader

Troubleshooting

Time Taken

Anticipated Results

References

Acknowledgements