Cytoprotein Extraction, Quantification & Western Blotting

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1 Introduction

2 Materials & Method

2.1 Cytoprotein extraction

Centrifuge, water bath, CO_2 incubator, hood, orbital shaker, pipttes and tips, cuvette, forceps, tissue culture dish, 15mL centrifuge tube, 1.5 centrifuge tube, Pasteur pipette.

RPMI 1640 supplied with 10%FBS, FBS, staurosporine(1mM stock), Methanol, RIPA lysis buffer(Beyotime P0013B: 50mM tris(pH 7.4), 150mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, sodium orthovanadate, sodium fluorid, EDTA, leupeptin, etc), Jurkat cell.

Jurkat cells was seeded to a dish, treat cells with 1μM staurosporine for 3 hr as experiment group and set a well not treated as control(Prepared by teacher). $\longrightarrow$ Harvest cell to 15mL tube $\longrightarrow$ centrifuge at 250xg, 5min $\longrightarrow$ Discard suspernatant, then add 200 μL RIPA lysis buffer and mix $\longrightarrow$ Incubate tube on ice for 30 min $\longrightarrow$ Transfer the cell lysate to 1.5mL tube $\longrightarrow$ Centrifuge 1300xg, 4°C $\longrightarrow$ Transfer suspernatant to new 1.5mL tube.

2.2 Cytoprotein quantification

Spectrophometer(Dynamica HALO XB-10), BCA protein quantification kit(Beyotime, P0009), PSA.

First, we made the standard curve of different concentration BSA solution as followin table. BCA solution should be added together in the end.

	S_0	S_1	S_2	S_3	S_4	S_5	S_6	S_7	Ctrl	Exp
PBS(5X)	100	95	90	85	80	75	70	65	95	95
0.5mg/mL BSA(μL)	0	5	10	15	20	25	30	35		
sample(μL)									5	5
BCA work solution(μL)	900	900	900	900	900	900	900	900	900	900

Table 1. Sample for BSA standard curve.

Then, all the tube were incubated in 60°C water bath for 30min. We cooled down all sample to room tempeture and briefly spin down. All sample are transfer to clean cuvtte and measure the absorbation at 562nm in spectrophometer after using S_0 to blank. Form BSA absorbation data, we got the standard curve and concentration $c(\text{ctrl}), c(\text{exp})$ of cytoprotein of control group and experiment group.

2.3 Western blotting

First, 20μg(9.1μL) lysate of control group and experiment group were added to 1.5mL tube one by one, while RIPA was used to adjust final volume to 32μL. More, 8μL of 5X SDS-PAGE loadding buffer was added to make final concentration 0.5μg/μL. These 2 sample were boiled at 100°C for 5min and centrifuge and store at -20°C.

3 Results

1. The standard curve is

$$y = 0.03874x - 0.007$$

where y is the absorbation at 562nm, and x is the concentration of BSA solution.

4 Discussions

5 Contributions

6 Figures

S_0	S_1	S_2	S_3	S_4	S_5	S_6	S_7	Ctrl	Exp
0	0.077	0.205	0.275	0.383	0.475	0.554	0.689	0.446	0.417

Table 2. The absorbation at 562nm of samples.

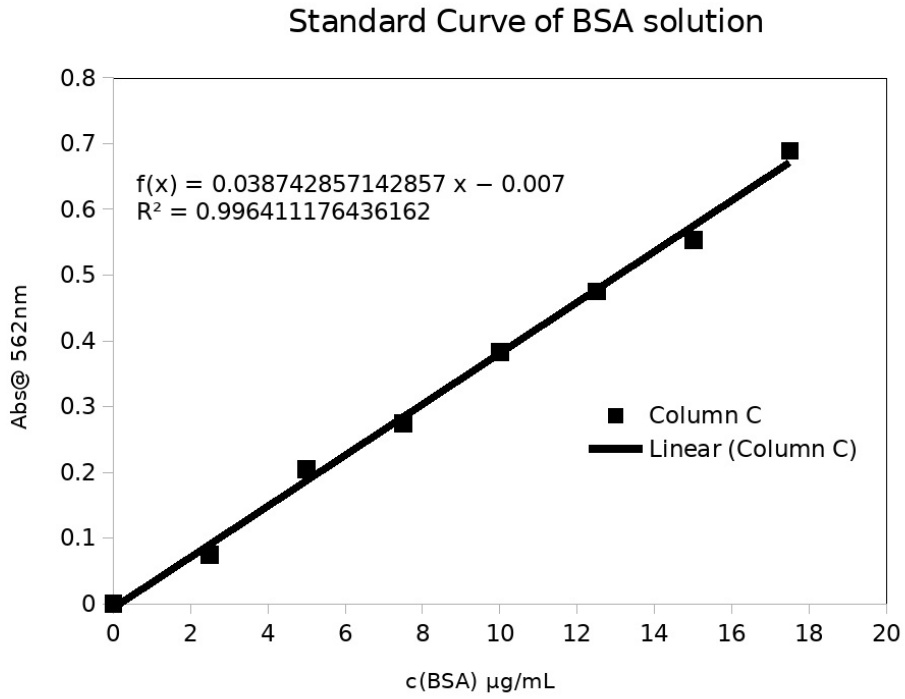

Figure 1. The standard curve of BSA solution.