Analysis of protein phosphorylation
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Analysis of protein phosphorylation is essential method of modern molecular biology. Many different phospho-specific antibodies are available. However, to be able to use these antibodies, one has to lyse the cells in a buffer that inhibits phosphatases, to preserve phosphorylation of proteins.

Searching the literature I found several different protocols and tried to generate efficient lysis buffer. It contains normal components of the lysis buffer, phosphatase inhibitors and protease inhibitors (Complete from Roche).

Since I usually need only small amount of the buffer I use stock solutions of all components and mix them, to get required volume of the buffer immediately prior to use. While this is not critical for most of the components, protease inhibitors and NaVO3 have to be added to the buffer immediately prior to use anyway.

The preparation of NaVO3 stock solution should be described additionally. I use sodium orthovanadate from Sigma (S6508). To get a stock solution prepare first a solution of about 300-400 mM in water and adjust pH to 10 with NaOH. The color of solution will change to yellow. Boil your solution, till it becomes colorless (takes only few minutes). Readjust pH to 10. If the solution becomes yellow again, repeat boiling. Adjust volume of your solution to get 200mM stock. You can find more information about sodium orthovanadate on Sigma web site.

Buffer formulation:

Tris HCl 50mM, pH 7.4,
EDTA 1mM,
NaCl 150mM,
NP40 1%,
NaF 5mM,
Na deoxycholate 0,25%,
add prior to use:
NaVO3 to 2 mM,
Protease inhibitors (Complete from Roche) to 1x

Stock solutions concentrations and volumes for 100 ml buffer

	Stock	Working	For 100 ml
Tris	1M	50mM	5 ml
EDTA	0,5 M	1 mM	0,2ml
NaCl	5M	150mM	3 ml
NP40	10%	1%	10ml
NaVO3	200mM	2mM	1 ml*
NaF	500mM	5mM	1 ml
Na deoxycholate	2.5%	0,25%	10ml
Complete	7x	1x	14ml*
H2O			to 100 ml

* Add prior to use

To analyse protein phosphorylation lyse 1x106 cells in 50 µl of buffer. To obtained cell lysate add 100 µl Laemmli buffer and incubate the samples at 95°C for 5 minutes. The proteins can be further analysed using normal Western blotting approach. Sometimes it is advisable to add phosphatase inhibitors to electrophoresis buffers as well as to the buffers used for membrane processing.