054/7566

New Monospecific Antibodies for Studies of Akt Signaling Pathways in Huntington's Disease

Tomas Dobransky[1], Martina Madarova[1], Fabiola M. Ribeiro[2]
[1] DB Biotech, Kosice, Slovakia; [2] Departamento de Bioquimica e Imunologica, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

INTRODUCTION

Huntington's disease (HD) is a neurodegenerative disorder characterized by a polyglutamine (polyQ) extension at the N-terminus of principal protein marker – huntingtin (Htt). PolyQ huntingtin promotes the formation of protein aggregates resulting in dramatic neural cell loss of affected region of brain, mostly in the areas of caudate and putamen, and neocortical regions of brain. The changes in Htt metabolism leads to cognitive decline, motor problems and demonstration of psychiatric symptoms. It had been shown previously, that mGluR5-regulated signaling pathways can alter the progression of the pathology, where neuroprotective phosphorylation of Htt plays one of the most important roles. Activation of Akt1 kinase by phosphorylation, and downstream neuroprotective phosphorylation of Htt, preventing its aggregation and polyQ-mediated neuronal cell death, plays the crucial role in prevention of disease progression.

Here we report a development and production of a set of new, original monospecific antibodies, based on in vitro cloning technology, recognizing specifically Akt1 kinase, with the wide application for western blot, immunoprecipitation, immunocytochemistry, and flow cytometry. The three original monospecific antibodies detect the epitopes present at the N-terminus, C-terminus, and in the middle of mouse, human and rat Akt1 kinase, and detect the whole Akt1 protein. The fourth, phospho-serine-473 (pSer-473) antibody, detect only the activated, phosphorylated form of Akt1. The Ser-473 phosphorylation of Akt1 induces a neuroprotective phosphorylation of Htt. This antibody can recognize also the pSer-474 of mouse Akt2, and pSer-472 of mouse Akt3, respectively. The application of these antibodies in quantification of Akt1 expression and pSer-473-related activation by flow cytometry, allows the direct and fast measurements of this protein in suspension of cultured striatal neurons. This antibody is crucial for further studies of potential hierarchical phosphorylation of Ser-473 regulated by phosphorylation of other serine residues at the C-terminus of Akt1 (Ser-475, Ser-477), Akt2 (Ser-476, Ser-478), and Akt3 (Ser-474, Ser-476), in mouse model of HD ($Hdh^{Q111/Q111}$), which we apply for pursuing the studies.

Acknowledgements

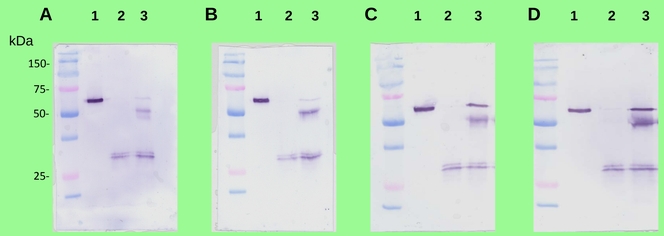
This work was supported by the grant from the Ministry of Education,Science, Research and Sport of the Slovak Republic to TD, DB Biotech, Slovakia and by the grant from CNPq-Brazil to FMR.

Rabbit Monospecific Clonal Antibodies

>sp|P31750|AKT1_MOUSE RAC-alpha serine/threonine-protein kinase OS=Mus musculus GN=Akt1 PE=1 SV=1
MNDVAIVKEGWLHKRGEYIKTWRPRYFLLKNDGTFIGYKERPQDVDQRESPLNNFSVAQC
QLMKTERPRPNTFIIRCLQWTTVIERTFHVETPEEREEWATAIQTVADGLKRQEEETMDF
RSGSPSDNSGAEEMEVSLAKPKHRVTMNEFEYLKLLGKGTFGKVILVKEKATGRYYAMKI
LKKEVIVAKDEVAHTLTENRVLQNSRHPFLTALKYSFQTHDRLCFVMEYANGGELFFHLS
RERVFSEDRARFYGAEIVSALDYLHSEKNVVYRDLKLENLMLDKDGHIKITDFGLCKEGI
KDGATMKTFCGTPEYLAPEVLEDNDYGRAVDWWGLGVVMYEMMCGRLPFYNQDHEKLFEL
ILMEEIAFPRTLGPEAKSLLSGLLKKDPTQRLGGGSEDAKEIMQHRFFANIVWQDVYEKK
LSPPFKPQVTSETDTRYFDEEFTAQMITITPPDQDDSMECVDSERRPHFPQFSYSASGTA

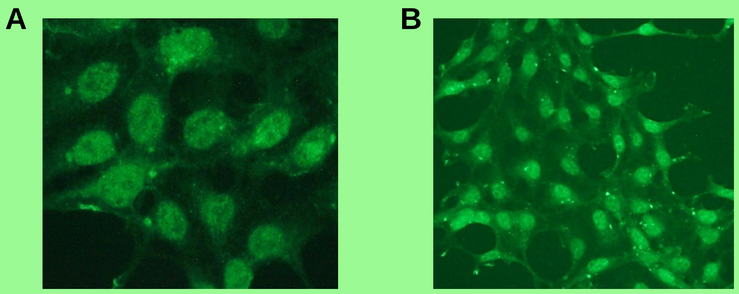
Original monospecific clonal anti-Akt1 antibodies
Four original antibodies for Akt1, Akt2 and Akt3 detection were designed and produced. Clones **E28-I** (detecting Akt1 and Akt2), **H20-I** detecting (Akt1, Akt2, Akt3), **C20-A** (detecting only Akt1 protein), and **X20-A**, recognizing pSer-473 of Akt1, pSer-474 of Akt2, and pSer-472 of Akt3, were produced by in vitro cloning technology, from the crude rabbit antiserum. All four clonal antibodies recognize human, mouse and rat Akt proteins.

Western Blot and Immunoprecipitation



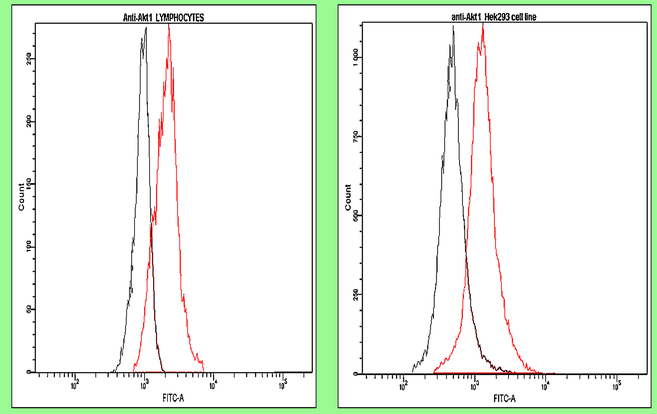
Akt was precipitated from crude protein extract of mouse striatal neurons with the clonal antibody: E28-I (A), H20-I (B), C20-A (C), and X20-A (D). Lane 1 … recombinant Akt1 protein; Lane 2 … immunoprecipitation without Akt antibody; Lane 3 … complete immunoprecipitation. The signal was detected with the clone C20-A, anti-Akt1 antibody

Immunocytochemisty of Akt1 in HEK 293 Cells



Endogenous Akt1 was detected in HEK293 cells with monospecific clonal anti-Akt1 antibody (clone C20-A). Distribution of Akt kinase is mostly nuclear (higher magnification, **A**). The lower distribution is obvious in cytoplasm, as shown in **B** (lower magnification).

Detection of Akt Kinase by Flow Cytometry



Whole blood permeabilized lymphocytes (A) or HEK 293 cells (B) were stained with FITC-conjugated Akt1 (red) specific antibody (clone C20-A) , with an isotype negative control (black)

Clustal W Alignment of Mouse Akt1, Akt2 and Akt3

```
CLUSTAL W (1.83) multiple sequence alignment
sp|P31750|AKT1_MOUSE      MNDVAIVKEGWLHKRGEYIKTWRPRYFLLKNDGTFIGYKERPQDVDQRESPLNNFSVAQC
sp|Q60823|AKT2_MOUSE      MNEVSVIKEGWLHKRGEYIKTWRPRYFLLKSDGSFIGYKERPEAPDQTLPPLNNFSVAEC
sp|Q9WUA6|AKT3_MOUSE      MSDVTIVKEGWVQKRGEYIKNWRPRYFLLKTDGSFIGYKEKPQDVDLP-YPLNNFSVAKC
                          *.:*:::****::*******.*********.**:******:*:  *    ********:*
sp|P31750|AKT1_MOUSE      QLMKTERPRPNTFIIRCLQWTTVIERTFHVETPEEREEWATAIQTVADGLKRQE--EETM
sp|Q60823|AKT2_MOUSE      QLMKTERPRPNTFVIRCLQWTTVIERTFHVDSPDEREEWMRAIQMVANSLKQRGPGEDAM
sp|Q9WUA6|AKT3_MOUSE      QLMKTERPKPNTFIIRCLQWTTVIERTFHVDTPEEREEWTEAIQAVADRLQRQE--EERM
                          ********:****:*:*****************:;*:*****  *** **:  *::;    *:  *
sp|P31750|AKT1_MOUSE      DFRSGSPSDNSGAEEMEVSLAKPKHRVTMNEFEYLKLLGKGTFGKVILVKEKATGRYYAM
sp|Q60823|AKT2_MOUSE      DYKCGSPSDSSTSEMMEVAVNKARAKVTMNDFDYLKLLGKGTFGKVILVREKATGRYYAM
sp|Q9WUA6|AKT3_MOUSE      NCSPTSQIDNIGEEEMDASTTHHKR-KTMNDFDYLKLLGKGTFGKVILVREKASGKYYAM
                          :    *  *.   * *:.:  : :   ***:*:****************:***:*:****
sp|P31750|AKT1_MOUSE      KILKKEVIVAKDEVAHTLTENRVLQNSRHPFLTALKYSFQTHDRLCFVMEYANGGELFFH
sp|Q60823|AKT2_MOUSE      KILRKEVIIAKDEVAHTVTESRVLQNTRHPFLTALKYAFQTHDRLCFVMEYANGGELFFH
sp|Q9WUA6|AKT3_MOUSE      KILKKEVIIAKDEVAHTLTESRVLKNTRHPFLTSLKYSFQTKDRLCFVMEYVNGGELFFH
                          ***:****:*********:**.***:*:******:***:***:*********.********
sp|P31750|AKT1_MOUSE      LSRERVFSEDRARFYGAEIVSALDYLHSEKNVVYRDLKLENLMLDKDGHIKITDFGLCKE
sp|Q60823|AKT2_MOUSE      LSRERVFTEDRARFYGAEIVSALEYLHSR-DVVYRDIKLENLMLDKDGHIKITDFGLCKE
sp|Q9WUA6|AKT3_MOUSE      LSRERVFSEDRTRFYGAEIVSALDYLHSG-KIVYRDLKLENLMLDKDGHIKITDFGLCKE
                          ******:***:*:*********:****   .:****:*********************
sp|P31750|AKT1_MOUSE      GIKDGATMKTFCGTPEYLAPEVLEDNDYGRAVDWWGLGVVMYEMMCGRLPFYNQDHEKLF
sp|Q60823|AKT2_MOUSE      GISDGATMKTFCGTPEYLAPEVLEDNDYGRAVDWWGLGVVMYEMMCGRLPFYNQDHERLF
sp|Q9WUA6|AKT3_MOUSE      GITDAATMKTFCGTPEYLAPEVLEDNDYGRAVDWWGLGVVMYEMMCGRLPFYNQDHEKLF
                          **.*.**********************************************:**
sp|P31750|AKT1_MOUSE      ELILMEEIAFPRTLGPEAKSLLSGLLKKDPTQRLGGGSEDAKEIMQHRFFANIVWQDVYE
sp|Q60823|AKT2_MOUSE      ELILMEEIRFPRTLGPEAKSLLAGLLKKDPKQRLGGGPSDAKEVMEHRFFLSINWQDVVQ
sp|Q9WUA6|AKT3_MOUSE      ELILMEDIKFPRTLSSDAKSLLSGLLIKDPNKRLGGGPDDAKEIMRHSFFSGVNWQDVYD
                          ******:*:*****:.:*****:*** ***:*****:.****:*.* ** .: *** :
sp|P31750|AKT1_MOUSE      KKLSPPFKPQVTSETDTRYFDEEFTAQMITITPPD--QDDSMECVDSERRPHFPQFSYSA
sp|Q60823|AKT2_MOUSE      KKLLPPFKPQVTSEVDTRYFDDEFTAQSITITPPD--RYDSLDPLELDQRTHFPQFSYSA
sp|Q9WUA6|AKT3_MOUSE      KKLVPPFKPQVTSETDTRYFDEEFTAQTITITPPEKYDDDGMDGMDNERRPHFPQFSYSA
                          *** **********.******:***** ******:    *.:: :: ::*.*********
sp|P31750|AKT1_MOUSE      SGTA
sp
sp
```

The regulatory phosphorylation of Ser-473 (Akt1), Ser 474 (Akt2), and Ser-472 (Akt3) is regulated in hierarchical manner by phosphorylation of other two serine residues at the C-terminus. The serine residues Ser-475 and Ser-477 (Akt1), Ser-476 and Ser-478 (Akt2), Ser-474 and Ser-476 (Akt3) are the targets for multiple protein kinases.

Conclusion

- New rabbit monospecific clonal antibodies recognize Akt1, Akt2 and Akt3 protein kinases, and can be applied for detection of human, mouse and rat proteins
- All four antibodies may be used for western blot and immunoprecipitation applications. Moreover, the clone C20-A may be applied for flow cytometry – when using the whole blood samples or adherent cell lines - and for immunocytochemistry