

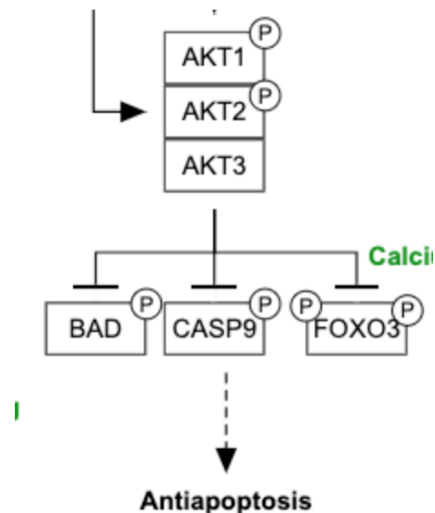
Modeling Phosphorylation Site Information in WikiPathways

These instructions describe how to use public databases to find relevant phosphorylation site information in the context of a pathway, for the purpose of annotating pathway nodes with the information as a structured comment. This annotation enables data mapping in tools like [Cytoscape](#).

The format of the structured comment is as follows, using the example below:

```
parentid=Q92934; parentsymbol=BAD; site=PFrGrsRsAPPNLWA; position=ser99; sitegrpId=447862; ptm=p; direction=d
```

The instructions use an example phosphorylation event from a larger pathway; the phosphorylation of the BAD protein by AKT1/2/3, which causes inactivation of BAD, ultimately leading to an anti-apoptotic effect.



1. Navigate to [PhosphoSitePlus](#) and search **Protein Name** "BAD". In the list of results, select the result for BAD, with gene name BAD.
2. In the **Protein Information** tab, record:
 - **parentid:** Q92934 (UniProt ID)
 - **parentsymbol:** BAD (Gene Symbol)
3. In the **Upstream** tab, look for Akt1/2/3 listed under **Kinase, *in vitro* or Putative in vivo kinase**. In this case, S99-p is listed as one such site.
4. For each site, open the site-specific page and record the following information:
 - **position:** Ser99
 - **sitegrpId:** 447862 (**Site Group ID**)

Also confirm that the **Effects of modification on biological processes** makes sense in the context of the pathway.

Home > Phosphorylation Site Page: > **Ser99 - BAD (human)**

Site Information

PFRGRSAPPNLWA [SwissProt](#) [Entrez-Gene](#)

[Blast this site against:](#) [NCBI](#) [SwissProt](#) [PDB](#)

Site Group ID: 447862

[Associated spectra:](#) [2 CST](#)

In vivo Characterization

Methods used to characterize site *in vivo*: [\[32P\] bio-synthetic labeling \(281 \)](#), [immunoassay \(74 \)](#), [immunoprecipitation \(62 , 70 , 286 , 288 , 291 \)](#), [mass spectrometry \(4 , 5 , 7 , 8 , 9 , 11 , 13 , 14 , 15 , 16 , 17 , 18 , 19 , view more >](#)

Disease tissue studied: [bone cancer \(30 \)](#), [brain cancer \(62 \)](#), [glioblastoma \(62 \)](#), [glioblastoma multiforme \(62 \)](#), [glioma \(62 \)](#), [breast cancer \(2 , 10 , 14 , 15 , 24 , 25 , 241 , 265 , 268 , 274 \)](#), [breast view more >](#)

Relevant cell line - cell type - tissue: ['neuron, cerebellar granule'-brain \(291 \)](#), ['pancreatic, ductal'-pancreas \(18 \)](#), ['stem, embryonic' \(165 \)](#), [293 \(epithelial\) \[AT1 \(human\), transfection, AT1R stable transfected view more >](#)

Upstream Regulation

Regulatory protein: [Akt1 \(human\) \(27 , 30 , 289 \)](#), [CDC42 iso1 \(human\) \(261 \)](#), [FLT3 \(human\) \(229 \)](#), [GRB10 \(human\) \(241 \)](#), [LAPTM4B \(human\) \(12 \)](#), [LKB1 \(human\) \(220 \)](#), [PAK1 \(human\) view more >](#)

Putative in vivo kinases: [Akt1 \(human\) \(141 \)](#), [BRAF \(human\) \(141 \)](#), [PAK1 \(human\) \(141 , 290 \)](#), [PKACA \(human\) \(141 \)](#), [PKCI \(human\) \(62 , 269 \)](#), [RAF1 \(human\) \(141 \)](#)

Kinases, *in vitro*: [Akt1 \(human\) \(70 , 141 , 286 , 291 \)](#), [ARAF \(human\) \(141 \)](#), [BRAF \(human\) \(141 \)](#), [PAK1 \(human\) \(141 , 290 \)](#), [PKCE \(human\) \(137 \)](#), [PKCI \(human\) \(62 , 269 \)](#), [PKCT view more >](#)

Treatments: [15-epi-LXA4 \(242 \)](#), [2-deoxyglucose \(158 \)](#), [AG1478 \(72 , 261 \)](#), [Akt_inhibitor_VIII \(72 \)](#), [Akt_inhibitor_X \(27 \)](#), [alkaline pH \(3 \)](#), [antibody \(10 , 283 \)](#), [carbachol \(27 \)](#), [view more >](#)

Downstream Regulation

Effects of modification on BAD: [activity, induced \(278 , 279 \)](#), [molecular association, regulation \(62 , 70 , 78 , 141 , 290 \)](#), [phosphorylation \(70 \)](#)

Effects of modification on biological processes: [apoptosis, altered \(99 , 220 , 274 , 289 \)](#), [apoptosis, inhibited \(3 , 59 , 278 , 288 , 290 \)](#), [cell growth, altered \(278 \)](#), [transcription, inhibited \(239 \)](#)

Induce interaction with: [14-3-3 beta \(human\) \(62 , 78 , 290 \)](#), [14-3-3 zeta \(human\) \(141 \)](#)

Inhibit interaction with: [14-3-3 beta \(human\) \(70 \)](#), [Bcl-2 \(human\) \(78 , 290 \)](#), [Bcl-xL \(human\) \(62 , 290 \)](#)

Disease / Diagnostics Relevance

Relevant diseases: [breast adenocarcinoma \(10 \)](#), [HNSCC \(74 \)](#), [ovarian cancer \(59 \)](#)

- Back on the main page for the protein, go to the **Site Table** tab and find the relevant site (S99 in this case). Record the **site sequence**, i.e. PFRGRSAPPNLWA.
- The **direction** parameter describes the effect that the phosphorylation has on the parent protein (BAD). This can be somewhat tricky to decipher from PhosphoSitePlus, so for this we use the direction indicated in the pathway model. In this case, there is an inhibition interaction between AKT1/2/3 and BAD, indicating the direction is a down-regulation. We will record this as “d” for **direction**.
- Construct the custom comment using the recorded information:

`parentid=Q92934; parentsymbol=BAD; site=PFRGRSAPPNLWA; position=ser99; sitegrp=447862; ptm=p; direction=d`

Note: If the information available from PhosphoSitePlus is not conclusive or clear, simply skip that site.