

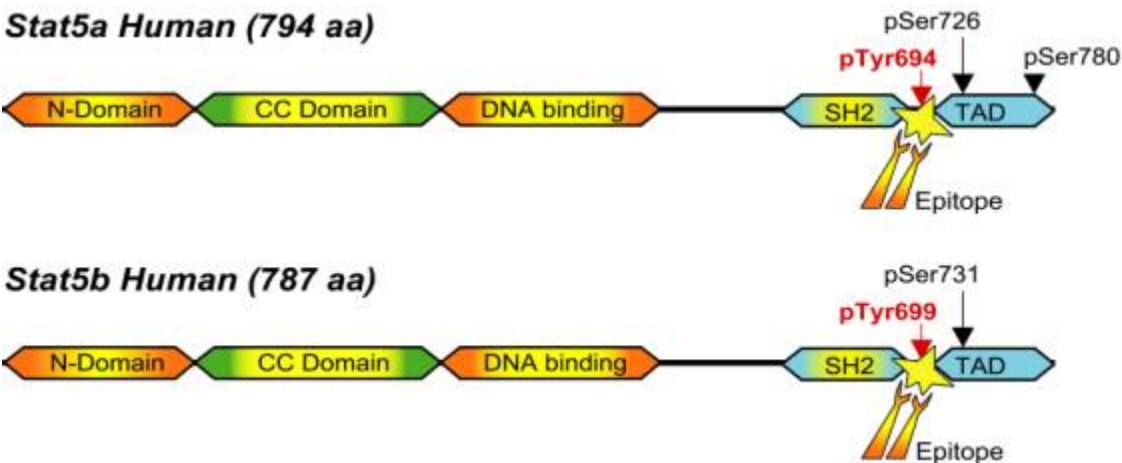
**AX1 antiphosphoStat5a/b(Y694/99) mouse monoclonal antibody**  
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Lyophilized 50 µg of antibody. Store at 4 C or below. Restore in 50 µl distilled water. Contains 0.05% sodium azide as a preservative.

**Applications:**

- \* **Immunohisto/cytochemistry:** (0.4-1.2 µg/ml, 16-20 h incubation)
- \* **Western Blot:** (0.2-1.2 µg/ml/ml, 16-20 h incubation)
- \* **Immunoprecipitation:** Not tested
- \* **Supershift analysis:** Not tested

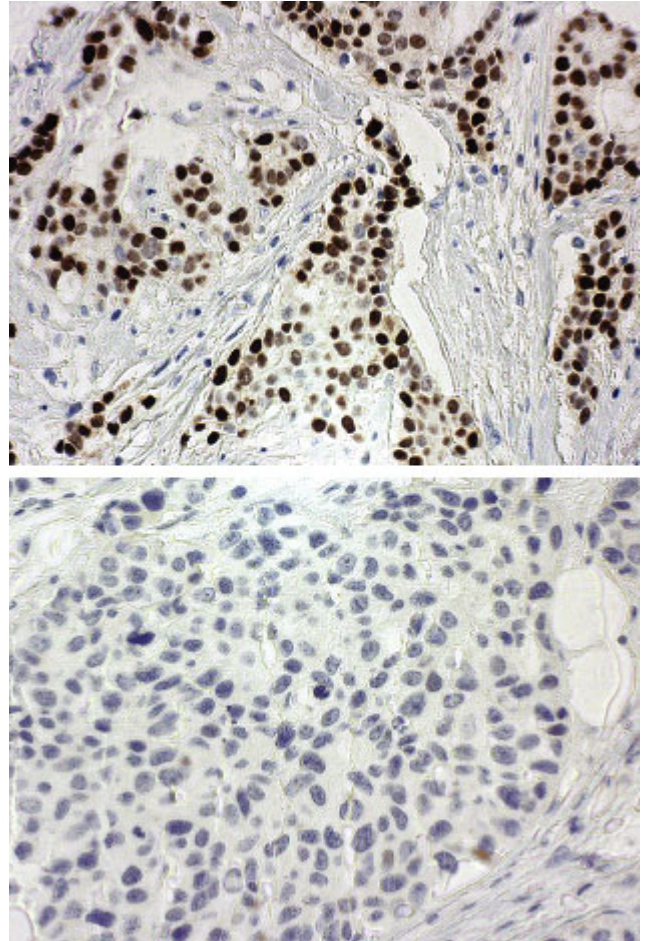
**Brief description of antigen:**Stat5a and Stat5b are transcription factors activated by peptide hormones, growth factors and cytokines, including prolactin, growth hormone, EPO, TPO, CM-CSF, interleukins, and EGF. Stat5a and Stat5b are activated by phosphorylation of conserved tyrosine residues Y694 and Y699, respectively, followed by nuclear translocation.



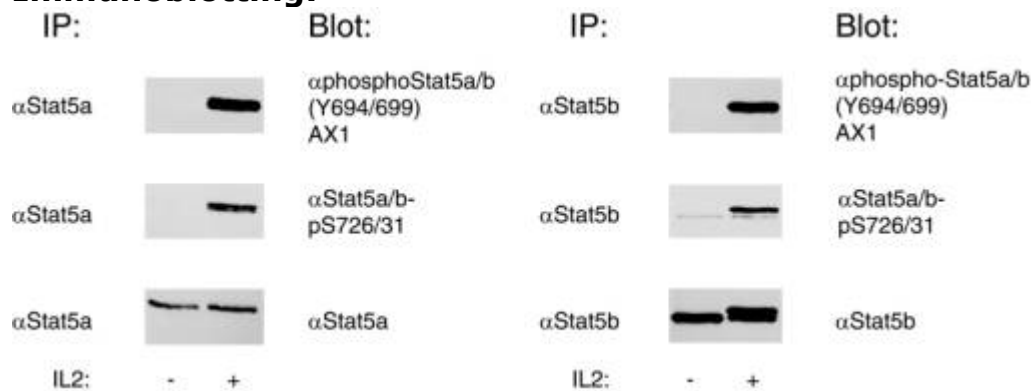
Immunogen	KAVDG [pY] VPQIK	Immunoreactivity
Human Stat5a	<sup>689</sup> KAVDG [pY] VPQIK <sup>699</sup>	Verified +
Human Stat5b	<sup>694</sup> KAVDG [pY] VPQIK <sup>704</sup>	Verified +
Mouse Stat5a	<sup>689</sup> KAVDG [pY] VPQIK <sup>699</sup>	Verified +
Mouse Stat5b	<sup>694</sup> KAVDG [pY] VPQIK <sup>704</sup>	Verified +
Rat Stat5a	<sup>689</sup> KAVDG [pY] VPQIK <sup>699</sup>	Verified +
Rat Stat5b	<sup>694</sup> KAVDG [pY] VPQIK <sup>704</sup>	Verified +

## Immunohistochemistry

Detection of active Stat5 in tissue sections. Breast cancer specimen (ductal breast carcinoma) with strong nuclear AX1 antiphosphoStat5 staining reflecting active Stat5 (upper panel), and a ductal breast carcinoma lacking active Stat5 (lower panel).

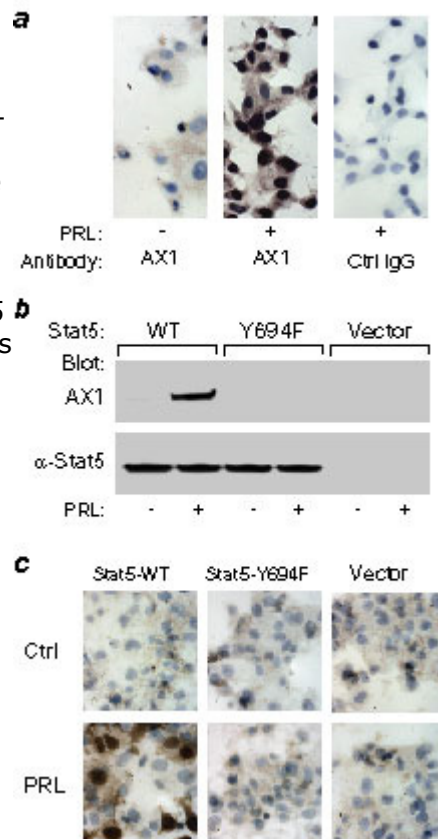


## Immunoblotting:



## Immunocytochemistry:

Antibody AX1 recognizes tyrosine phosphorylated Stat5 in formalin-fixed cells. a) Immunocytochemistry of T47D cells. Human T47D breast cancer cells incubated with (+) or without (-) human prolactin (PRL) were analyzed for tyrosine phosphorylated Stat5 by immunocytochemistry using AX1. Note strong staining in nuclei of prolactin-stimulated cells (middle panel). b) Immunoblotting of Stat5 in COS-7 cells expressing WT or phosphorylation-defective Stat5. COS-7 cells transiently transfected with plasmids encoding prolactin receptors and Stat5 were incubated in the presence or absence of prolactin. The cells were lysed and Stat5 was immunoprecipitated, separated on SDS-PAGE and parallel samples were either blotted with anti-pTyr-Stat5 antibody AX1 (upper panel) or anti-Stat5 antibody (lower panel). Consistent with specificity of recognition of the critical phosphorylation site of Stat5, antibody AX1 recognized prolactin-induced tyrosine phosphorylation of WT-Stat5a but not of mutant Stat5a-Y694F, which lacks the phosphoacceptor hydroxyl group. c) Immunocytochemistry of COS-7 cells expressing WT or phosphorylation-defective Stat5. COS-7 cells transfected and treated as above (b) were stained with antibody AX1 for detection of inducible tyrosine phosphorylation of Stat5. Consistent with specificity of recognition of the key phosphotyrosine residue of Stat5, AX1 recognized prolactin-induced tyrosine phosphorylation only in cells transfected with WT-Stat5a (top right panel) but not in cells transfected with the phosphorylation-defective mutant Stat5a-Y694F (middle panels) or in untransfected cells (bottom panels).



## Immunohisto/cytochemistry protocol:

Use at 0.4-1.2  $\mu$ g/ml, 1-20 h incubation (Increased sensitivity with the higher antibody concentrations and time).

For best results use antigen retrieval by heating in microwave or in waterbath (95 C, 20 min; retrieval buffer AXAR1 (Advantex Bio) or other antigen retrieval buffers). To avoid loss of tissue during antigen retrieval, use highly adhesive glass slides (positively charged "++" slides (e.g. Fischer Scientific)) [for even greater adhesiveness, consider Instrumedics, Inc [Paraffin Tape-Transfer Slides 4+](#)]. An amplification system between at the secondary antibody step is recommended, eg. BioGenex "Link and Label", or other similar products. For immunocytochemistry, heating at 95 C for 20 min in retrieval buffer in a water bath is preferred over microwaving.

## Application References:

Johansson et al, Nuclear Factor 1-C2 Is Regulated by Prolactin and Shows a Distinct Expression Pattern in the Mouse Mammary Epithelial Cells during Development. [Mol Endocrinol 19: 992-1003, 2005.](#)

Sultan et al, Stat5 promotes homotypic adhesion and inhibits invasive characteristics of human breast cancer cells. [Oncogene, 24:746-60., 2005.](#)

Nevalainen MT et al. Signal Transducer and Activator of Transcription-5 activation and breast cancer prognosis. [Journal of Clinical Oncology, 22, 2053-2060, 2004.](#)

Wagner K-U, et al. Impaired alveolar specification and maintenance of secretory mammary epithelial cells in Jak2 conditional knockout mice. [Molecular Cell Biology, 24, 5510-5520, 2004.](#)

Nevalainen et al, Basal activation of transcription factor Stat5 in nonpregnant mouse and human breast epithelium. [Molecular Endocrinology, 16, 1108-1124, 2002.](#)

Ahonen et al, Inhibition of Transcription Factor Stat5 Induces Cell Death of Human Prostate Cancer Cells. [Journal of Biological Chemistry, 278: 27287-92, 2003](#)

Li et al, Mammary Gland Development in Transgenic Male Mice Expressing Human P450 Aromatase. [Endocrinology, 143, 4074-4083, 2002.](#)

Ahonen et al, Prolactin signal transduction in the epithelial compartment of rat prostate maintained as long-term organ cultures in vitro. [Endocrinology, 143:228-238, 2002.](#)

Xie et al, Role of tyrosine kinase Jak2 in prolactin-induced differentiation and growth of mammary epithelial cells. [Journal of Biological Chemistry, 277, 14020-14030, 2002.](#)

**Review:** J. W. Mandell. **Phosphorylation State-Specific Antibodies: Applications in Investigative and Diagnostic Pathology.** [Am. J. Pathol., November 1, 2003; 163\(5\): 1687 - 1698.](#)

## Key Benefits

- 🟡 Works in formalin-fixed, paraffin-embedded tissue section
- 🟡 Does not cross-react with unphosphorylated Stat5 ([see ref 1](#))
- 🟡 Allows specific detection of activated Stat5a/b at single cell resolution
- 🟡 Less than \$ 1 per test (enough for 500 immunohistochemical tests)

## Pricing

Description	SKU #	Price
50 µg	AX1	499

Sufficient for at least 500 immunohistochemical slides!

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