

## BACKGROUND

The aminoglycoside phosphotransferases belong to a clinically important group of proteins that enable bacteria to grow in the presence of selected aminoglycoside antibiotics, including kanamycin, neomycin, and G418. These enzymes, which are plasmid or transposon encoded, inactivate the antibiotics by catalyzing the transfer of the terminal phosphate of ATP to the drug.<sup>1</sup> Two aminoglycoside phosphotransferase genes are used in selection of transformed organisms: the neomycin phosphotransferase I (*nptI*) gene and the neomycin phosphotransferase II (*nptII*) gene. Neomycin phosphotransferase II (NPTII; EC 2.7.1.95) is the most widely used selectable marker in the transformation of organisms including yeast, *Dictyostelium discoideum*, mammals, and plants. NPTII was initially isolated from the transposon Tn5 that was present in the bacterium strain *Escherichia coli* K12. NPTII is also used in gene expression and regulation studies in different organisms in part because N-terminal fusions can be constructed that retain enzymatic activity. In animal cells, G418 and neomycin are used as selectable agents.<sup>2</sup> The gene is usually modified to contain a promoter and terminator that can function in the recipient host. Cells that receive and properly express such chimeric constructs can be selected by using antibiotic concentrations that inhibit the growth of cells not containing the resistance gene. The aminoglycoside 3'-phosphotransferases exhibit 30-36% amino acid sequence homology except in a region near the carboxyl terminus, where the homology is 60-70%. It has been suggested that the catalytic functions of binding ATP and transferring the terminal phosphate are performed at the more conserved region. The mutation causes reduced NPTII enzyme activity, resulting in a decreased ability of transformants to grow in the presence of elevated concentrations of antibiotic.<sup>3</sup>

### References:

1. Wright, G.D. & Thompson, P.R.: Front. Biosci. 4:d9-21, 1999
2. Argyle, J.L. et al: Appl. Environ. Microbiol. 62:4233-7, 1996
3. Yenofsky, R.L. et al: Proc. Natl. Acad. Sci. USA 87:3435-9, 1990

## TECHNICAL INFORMATION

### Source:

NPTII Antibody is a mouse monoclonal antibody raised against recombinant human NPTII fragments expressed in *E. coli*.

### Specificity and Sensitivity:

This antibody detects NPTII proteins without cross-reactivity with other family members.

**Storage Buffer:** PBS and 30% glycerol

### Storage:

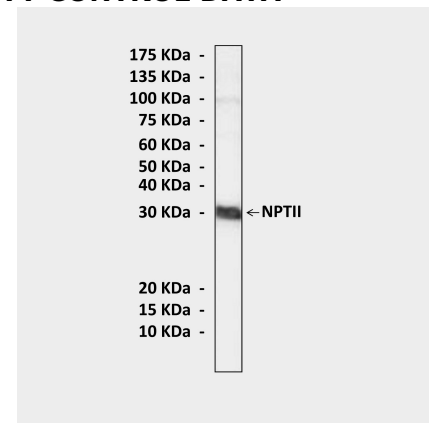
Store at -20°C for at least one year. Store at 4°C for frequent use. Avoid repeated freeze-thaw cycles.

## APPLICATIONS

Application:	*Dilution:
WB	1:1000
IP	1:50
IHC	n/d
ICC	n/d
FACS	n/d

*\*Optimal dilutions must be determined by end user.*

## QUALITY CONTROL DATA



Western Blot detection of recombinant NPTII proteins expressed in *E. coli* cells using NPTII Antibody.

