



POLYCLONAL ANTIBODIES AGAINST SYNTHETIC PHOSPHO-PEPTIDE

This service is useful for the development of polyclonal antibodies able to recognize the presence of phosphorylation in proteins samples. The antigen used is a phosphorylated synthetic peptide carrying the amino acid sequence of the phosphorylation site of the antigen protein.

According to our standard procedure, **Phospho Specific Polyclonal Antibody** production includes use of two **rabbits** immunized with a phospho-synthetic peptide conjugated to either OVA or KLH.

Additional synthesis of the corresponding non phosphorylated peptide is required to carry out the differential purification of phospho-specific antibodies and for ELISA test.

- Free of charge selection of the most immunogenic sequence carrying the phosphorylated residue

STANDARD PROTOCOL FOR IMMUNIZATION WITH PHOSPHO SYNTHETIC PEPTIDE:

IMMUNIZATION TIME	RABBIT
Preimmune serum collection	1 ml/rabbit
T0 1st immunization	300 µg Ag in CFA
T21 2nd immunization	300 µg Ag in IFA
T28 3rd immunization	300 µg Ag in IFA
T35 4th immunization	300 µg Ag in IFA
T42 Bleed + ELISA* test	500 µl
T45 5th immunization	150 µg Ag in IFA
Final Bleed	40-50 ml/rabbit

*Serum positivity is guaranteed at a dilution of the serum equivalent to 1:10,000 as compared to pre-immune serum in ELISA test against the peptide antigen.

CFA Complete Freund Adjuvant

IFA Incomplete Freund Adjuvant



Phospho specific antibodies are purified by total immuno absorption of the immune serum using CNBr-Sepharose non-phospho-peptide column and finally affinity chromatography is performed using CNBr-Sepharose phospho peptide column (keeping separate the purified prep of each rabbit).

TIME REQUIRED:

3 months (3/4 weeks to synthesize peptides, 1.5/2 months to immunize animals and 1/2 weeks for immunoabsorption and immunoaffinity purification)

DELIVERED PRODUCTS:

DELIVERED PRODUCTS	RABBIT
Preimmune sera	1 ml/rabbit
Immune sera	1 ml/rabbit of immune serum + 2 preps of immunopurified phospho-specific polyclonal antibody (+ ELISA test profile)
Free phospho-peptide	3 mg
Free non-phospho-peptide	3 mg

ADDITIONAL SERVICES (at additional charge):

ADDITIONAL SERVICES	RABBIT
Testing of 5 or 10 preimmune sera to select animals before immunization	yes
Prolongation of immunization protocol	yes