

## Conjugates of Secondary antibodies, Protein A, Avidin, Streptavidin or Biotin

### Secondary antibody conjugates:

Secondary antibodies used in the conjugation are purified by immuno-affinity chromatography using antigen linked Sepharose®. This process yields antibodies with high avidity and minimum cross-reaction with other molecules. Conjugates of these antibodies react only with immunoglobulin-G.

### Conjugates absorbed with other species serum proteins:

Selected secondary antibodies have been subjected to additional purification by passing them through Sepharose coupled with serum proteins of other species. This additional step, minimizes the cross reactivity with immunoglobulins of other species.

### Conjugates of other proteins:

Protein A, avidin and streptavidin are conjugated with ALP, HRP and FITC and lectins are conjugated with FITC and biotin. Proteins having high activity and more than 95 % purity, are used for the conjugation.

### Conjugates for detection of recombinant proteins:

Please refer index

## Titration of the conjugates

### Direct ELISA (dELISA):

Direct ELISA is done using 0.5 µg/100 µl antigen coated wells and 100 µl diluted conjugates. The conjugate is allowed to bind for 30 minutes at room temperature and then bound conjugate is estimated by using appropriate substrate. Substrates used are p-nitrophenyl phosphate (Cat # 106039) 1 mg/ml in 1 M diethanolamine, for ALP and TMB/H<sub>2</sub>O<sub>2</sub> (Cat # 106035) or OPD 1 mg/ml in citrate phosphate buffer, for HRP. The dilution, which corresponds to an OD between 1.0 & 1.2 in ELISA reader is quoted as titre.

### Direct immunoblot development (DIBD):

20 ng of antigen is spotted on NC membrane blocked with 5 % skimmed milk. The strip is incubated with diluted conjugate, washed and developed with appropriate substrate. BCIP/NBT (Cat # 106036) is used for ALP and TMB/H<sub>2</sub>O<sub>2</sub> (Cat # 106037) is used for HRP. The maximum dilution of conjugate at which a visible antigen spot appeared is quoted as the titre.

### Western Blot (WB):

Western blot is done using antigen 10 µg/lane on SDS PAGE and blot development with appropriate conjugate and substrate. The maximum dilution of conjugate at which a visible antigen band appeared is quoted as the titre.

### Particulate immunofluorescent assay (PIFA):

The antigen immobilized cross-linked agarose beads are incubated with various dilutions of the conjugates, washed and viewed under fluorescent microscope. The maximum dilution of conjugate at which fluorescence of the beads is sufficient to take a clear picture is quoted as the titer.

## Enzyme Conjugates:

Label	ALP	HRP
Source	Calf intestinal mucosa	Horseradish
Activity	4000 - 5000 DEA units/mg	200 - 250 purpurogallin units/mg
Conjugation method	Maleimide	Periodate
Molar Incorporation	1 - 2	2 - 3
Antibody/protein conc.	1 - 1.5 mg/ml	0.8 - 1.2 mg/ml
Storage	4° C	4° C
Titre	dELISA 1:10000 to 1:15000	dELISA 1:15000 to 1:35000
	DIBD 1:10000 to 1:15000	DIBD 1:15000 to 1:20000
	WB 1:2000 to 1:3000	WB 1:2000 to 1:5000
Applications	ELISA, Blot development, Immunohistochemistry	ELISA, Blot development, Immunohistochemistry