PRODUCT DATA INFORMATION

DESCRIPTION : Protein A - Horseradish Peroxidase Conjugate (Prot. A-HRP)

CAT. NO. : HP-02

LOT NO. : 0915F

PURITY: Pure protein A and HRP used for conjugation

PROTEIN CONCENTRATION: ~~xxx/xxx~~ 1.82mg/2ml
214.4 units

OTHER ANALYTICAL DATA : Gel filtration was used to separate the conjugates from the non-conjugated HRP and protein A

CHEMICAL USED FOR CONJUGATION :

METHOD USED FOR CONJUGATION : Modified procidene of nakane method

OTHER TECHNICAL INFORMATION :

CAUTION : SO NOT EXPOSE TO STRONG LIGHT

REF.:

E-Y LABS INC. P.O.BOX 1787, SAN MATEO, CA 94401
TEL.(415) 342-3296, TLX 349336

HORSERADISH PEROXIDASE ENZYME ACTIVITY ASSAY

ACTION OF PEROXIDASE: Peroxidase + H_2O_2 → complex
complex + AH_2(donor) → Peroxidase + H_2O + A(colored)

ASSAY REAGENTS:
1. Enzyme: Dilute to obtain 1.2ug/ml with PBS. Acceptable dilution range: 1-2ug/ml.
2. Substrate: Stock H_2O_2. 1ml 30% H_2O_2 in 100ml H_2O. To use, dilute 1ml of the H_2O_2 (.3%) with 100ml .01M PBS pH 6.0 (fresh daily).
3. Buffer: 0.01M phosphate buffer, pH 6.0
4. Dye: 1% o-dianisidine in methanol made fresh in amber bottle.

PROCEDURE: Add 0.05ml of dye to 6.0ml of substrate. Transfer 2.9ml to reaction test tube and 2.9ml to control test tube. At zero time, add 100ul of enzyme dilution to reaction tube and 100ul PBS to control tube. Mix thoroughly. Record OD460 every 15 sec. for 3 min., or take only end point reading after 3 min. by stopping the reaction with 100λ concentrated NaN_3. Use this value to determine the rate of change in absorbance per min.

CALCULATIONS:

$$\Delta OD460/min = \frac{OD460/3min - ODcontrol/3min}{3min}$$

$$\text{mg enzyme/ml reaction mix.} = \frac{[\text{enzyme dilution}]}{30}$$

$$\text{units/mg} = \frac{\Delta OD460/min}{11.3^{*}x \text{ mg enzyme/ml reaction mixture}}$$

*$11.3 \times 10^3 cm^{-1}$ is molar absorbance of H_2O_2.
One unit of peroxidase activity is that amount of enzyme decomposing 1umole peroxide/min at 25°C.