3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

PROTEIN A-β-GALACTOSIDASE CONJUGATE

Product Number **P 7650**

Product Description
This conjugate of protein A, from Staphylococcus areus, and β-galactosidase, from Escherichia coli has the highest purity compounds chemically linked by glutaraldehyde in a molar ratio of approximately 3 protein A molecules to 1 β-galactosidase. Used in a 10^5 to 10^6 fold dilution, this conjugate provides extreme sensitivity in the determination of human IgG by using, as a measure of attached conjugate, the rate of hydrolysis of 4-methylumbelliferyl β-D-galactoside, determined fluorimetrically. Ten microliters of this P 7650 conjugate will serve for 3,000 to 30,000 single well tests.

Reagents
Supplied as a solution in 45% glycerol, 0.01 M potassium phosphate buffer, pH 7.3, containing 0.15 M sodium chloride, 1% bovine serum albumin, 10^{-6} M magnesium chloride, and 10 ppm 4-chloro-3,5-dimethylphenol (as preservative).

Storage/Stability
Store in freezer at –5 °C to –25 °C.

Procedure
Buffer: 0.05 M potassium phosphate, 0.15 M NaCl, 0.05% Tween 20, pH 7.4
IgG Solution: 2 µg/ml Human IgG (Product No. I 4506) in 0.10 M sodium carbonate buffer, pH 9.6
Conjugate Solution: 10^5 dilution (1µl to 100 ml) of Protein A-β-Galactosidase conjugate in buffer
Substrate Solution: 0.1 mM 4-Methylumbelliferyl β-D-Galactoside (Product No. M 1633, MW 338.3) in 0.1M potassium phosphate, pH 7.3, containing 0.15 M NaCl and 1 mM $MgCl_2$
Glycine Solution: 0.1 M Sodium Glycine, pH 10.4

1. Using polystyrene microplates, having a well volume of at least 3.5 ml, incubate 0.30 ml of IgG Solution in each well for 30 minutes at 37 °C, then overnight at 5 °C.
2. Empty the wells by shaking. Then rinse by filling with Buffer, incubating for 30 minutes at 25 °C and emptying wells. Repeat this rinsing two more times.
3. Attach conjugate by incubating 0.30 ml of Conjugate Solution in each well for 3 hours at 25 °C.
4. Wash each well three times as in Step 2.
5. Add 0.30 ml of Substrate Solution to each well and incubate for 30 minutes at 37 °C.
6. Stop the reaction by adding 2.7 ml of Glycine Solution to each well.
7. Determine the fluorescence of the solution in each well using a fluorometer with excitation at 360 nm and emission at 450 nm. As a blank, use a 1:10 dilution of Substrate Solution in Glycine Solution. As a standard, prepare a 1.0×10^{-7} M Methylumbelliferone, Sodium Salt, Solution in Buffer (Product No. M 1508, a 1:100 dilution of a 19.8 mg/100 ml solution). Then dilute 0.3 ml of the Methylumbelliferone Standard Solution with 2.7 ml of Glycine Solution and measure the fluorescence on an appropriate scale.

At the IgG Solution concentration given in this procedure, there are 4.0 picomoles of IgG present in each well. Using appropriate changes in incubation time (Step 5), standard concentration, and fluorescence scale, femtomole quantities of IgG could be determined.

NOTE: This conjugate may also be used in a much less sensitive colorimetric procedure with a 100-fold dilution of the conjugate and o-Nitrophenyl β-D-Galactoside (ONPG, Product N1127) as the substrate. Substrate solution contains 2 mM ONPG in 0.1 M potassium phosphate buffer, pH 7.3, containing 0.10 M 2-mercaptoethanol and 1 mM $MgCl_2$. The rest of the procedure remains the same through Step 6, and the absorbance at 405 nm is determined with a spectrophotometer.

References

Goudbard, S. B., et al., J. Immunol. Methods, **68**, 137-146 (1984).
Savelkoul, H. F., et al., J. Immunol. Methods, **116**, 277-285 (1989).
Van Soest, P. L, et al., Histochem J., **16**, 21-35 (1984).

JWM/KMR 04/02