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TRIPLE SANDWICH IMMUNOPRECIPITATION SYSTEM

Ag* -mMAb- rat anti-mouse Kappa chain MAb-Staph.aureus

GENERAL:

This is an indirect immunoprecipitation system for use with mouse MAb. Mouse immunoglonbulin subclasses bind to protein A on the surface of Staph. aureus bacteria through their Fc portion. The IgG's vary in their capability to bind depending on the heavy chain subclass. For example: IgG2a and IgG2b bind better than some IgG1.

Mouse MAb contain K light chain $\geq$ 90%. A rat anti-mouse Kappa chain MAb (187-1) [Yelton, D. E., C. Desaymard and M. D. Scharff, 'Use of monoclonal anti-mouse immunoglobulin to detect mouse antibodies'; Hybridoma 1: 5-11, 1981] is a useful reagent for most mouse MAb independently of their Ig subclass. This reagent is a rat IgG2c and binds to protein A. Excellent results can be obtained with the combination of both 187-1 MAb and Staph. aureus.

METHOD:

1) Preclearing of labeled cell lysates with Staph. aureus (SA). Before immunoprecipitation, labeled cell lysates in a 1.5 ml Sarstedt conical tube should be mixed and incubated with Staph. aureus bacteria (200 µl of a 10% suspension of SA per ml of cell lysates) to preclear the proteins that bind nonspecifically to SA. After shaking for 1 h at 4 ^{0}C, the mixture is centrifuged (2,000 rpm x 10 min.) and the supernatant can be used for immunoprecipitation.

Before use, SA is washed 3 times with and resuspended in TSA pH 7.4, 1% TX-100 and 0.1% glycerol.

2) Immunoprecipitation. Precoat 1.5 ml Sarstedt tubes without caps with 1% Hb in TSA. Add in order wash buffer (25 µl), 50-100 µl of mouse MAb culture supernatant (the equivalent to 10 µg) and mix with 10-100 µl labeled cell lysates containing 2-3 x 10^{5} cpm of ^{125}I-antigen. Vortex and allow to stand 2 h, at 4 ^{0}C. Then, add 100 µl/sample of rat anti-mouse Kappa chain 187-1 MAb culture supernatant ($\simeq$ 7.4 µg), vortex and allow to stand at 4 ^{0}C for 0.5 h or overnight. After the second incubation 50 µl of a 10% suspension of washed Staph. aureus per sample is added. Shake for 10 min. at 4 ^{0}C. Washing and later steps are as described in in 'Immunoprecipitation for Analysis of Radiolabeled Cell Surface Molecules.'