PROTOCOL

Protocol for CD54 (ICAM-1) FACS analysis using RR1/1-Purified (Prod. No. ALX-805-016):

$5x10^6$ cells of the investigated line (e.g. K562, Daudi) are suspended in 0.2ml RPMI-1640 and incubated for 30 minutes with 0.5µg of RR1/1 on ice. As a control, cells are incubated with an equal concentration of mouse antibody under the same conditions. Following incubation, cells are washed twice with 2ml PBS containing 0.1%BSA and centrifuged at 180 x g for 8 minutes. Cells are resuspended in 0.2ml RPMI-1640. 5µl (7.5µg) of a FITC labeled goat anti-mouse antibody are added, and samples are incubated for 30 minutes on ice. Following another two washing steps as described above, cells are resuspended in 0.5ml RPMI-1640 without phenol red, and stored on ice in the dark until measurement. Antibody binding is detected using EPIX CS at a wavelength of 488nm.

Protocol for adhesion blocking with RR1/1-Purified (Prod. No. ALX-805-016):

Experiments to block adhesion are normally done with radioactive labeled leukocytes or fluorescence labeled material. References for the radioactive procedure are: DiCorleto et al., Biochem. J. **264**, 71 (1981) and Zweimann et al., Cell. Immunol. 68, **165** (1982) (Fluorescence Method 1).

1) Antibody against CD54 (ICAM-1): Endothelial cells which express CD54 (ICAM-1) (confluent monolayer in a 24-well Falcon plate) are washed with warm culture medium (M199). Incubate with RR1/1-Purified at room temperature for 45-60 min. The antibody concentration should be as high as possible. The best way is to use F(ab')2 fragments to eliminate binding of antibody to Fc receptors (on granulocytes, NK cells, B cells and monocytes).
2) Labeling of lymphocytes: Lymphocytes ($1x10^7$/ml) are incubated with CSFE (carboxyfluorecein-succinimidylester). Use 0.5M CSFE in 0.5 x PBS as stock (the ratio is 1:10 related to lymphocytes). Incubate for 15 min. at 37°C. Wash twice with ice cold 1 x PBS.
3) Adhesion study: The CSFE labeled lymphocytes are added to the endothelial cells, labeled with RR1/1-Purified. The ratio should be 5-10:1 (lymphocytes:ec), i.e. ~1 x 10^6 cells/well. Incubate for 60 min. in M199 at 37°C containing 5% CO_2. Wash the non-adherent cells with warm medium with care. Add 0.5 ml warm PBS and measure fluorescence: Stimulate at 485 nm, emission at 530 nm. This test can also be used with lysates (lysate co-culture with 1M NaOH). Standard curve with dilutions of CSFE labeled lymphocytes. Sensitivity up to 5 x 10^3 lymphcytes/well.

Other fluorescence dyes may be used: i.e. BCECF (Rice et al., J. Exp. Med. 171, 1369 (1990); Toyama-Sorimachi et al., Eur. J. Immunol. 23, **439** (1993); Vaporciyan et al., J. Immunol. Methods **159**, 93 (1993).

Western blot protocol for CD54 (ICAM-1) using RR1/1-Purified (Prod. No. ALX-805-016):

Protein that has been immobilized on nitro cellulose should be blocked overnight in 2% BSA-tris buffered saline at 4°C (10 mM TRIS-HCl, pH 8.0, 150 mM NaCl). The primary antibody RR1/1-Purified is diluted in TBST (TRIS-buffered saline with 0.05% Tween 20) to a concentration of 0.5-5 µg/ml. Remove blocking solution and incubate with primary antibody for 30 min. at room temperature on a shaking platform. Wash the nitrocellulose three times with TBST for 5-10 min. each to remove unbound antibody. The secondary antibody against mouse Ig should now be added as per the manufacturers instructions. There are generally good results with alkaline phosphate conjugates.

The procedures listed above are intended only as a guide. Various assay conditions require that the investigator determine the optimal working concentrations. The results may vary depending on experimental conditions and technique. No warranty or guarantee of performance of above procedure is made or implied. Use good laboratory practices and handle all materials with care.

These products and procedures are for in vitro experimental use only and are not intended for use in humans or clinical diagnosis.