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B cell blind panel transfectant results

CD19	110
CD20	111
CD22	48, 55
CD23	78, 83
CD37	none
CD38	95, 106
CD39	79, 99
CD40	18

CDS reactive	40, 44, 66, 71, 73, 75, 80, 81, 84, 85, 92, 97, 100, 104, 112, 127
Not tested (absent)	10S,130-141(inclusive)

CDS cells were transfected with the cDNA expression clones listed above in 10 cm dishes using DEAE Dextran as a facilitator. The cells were trypsinized the day prior to transfection and spl it 1:8 into dishes containing 6 ml of DME/10% NuSerum/15 ug/ml gentamicin sulfate. On the day of transfection DNAs were added to each dish to 100-300 ng/ml, followed by 0.25 ml of a solution containing DEAE Dextran (10 mg/ml) and Chloroquine (2.5 mM). After incubation at 37 C for 4 hours the medium was aspirated and a solution of 10% DMSO in PBS was added for at least 2 minutes at room temperature. The DMSO solution was aspirated, 6 ml of DME/NuSerum/gentamicin added and the cells were returned to the incubator. 12-24 hours later the cells from each plate were removed with trypsin and passaged onto two sulfonated 96 well I culture plate lids in 10 ml of medium per lid. After incubation for 2-3 days the lids were washed with 0.15 M NaCl, fixed with cold (-20 C) methanol : acetone 7:3, air dried, and stored.

For immunofluorescence analysis the lids were wet with PBS/5% Calf Serum/0.5 mM EDTA (PBS/CS/EDTA), excess fluid removed, and 20 micro liters of a 1: 400 dilution of anti body in PBS/CS/EDTA were added. After incubation for greater than 45 minutes, the lids were washed with Q.15 M NaCI, rewet with PBS/CS/EDTA, the excess fluid removed, and 25 microliters of a 1:100 to 1:200 dilution of fluorescein-conjugated Goat anti-Mouse IgG+IgA+IgM (Cappel) in PBS/CS/EDTA were added. After Incubation for greater than 45 minutes, the lids were washed with 0.15 M NaCI and examined under alternating phase and epifluorescent illumination with a Zeiss Universal microscope and a 2SX oil/glycerol/water immersion objective (Plan-NEOFLUAR) immersed in 0.15 M NaCl.

Each antibody was tested on each of the transfectants listed above. Posititive and questionable signals were confirmed by retesting on new lids in the same manner.