MICROTITER STAINING METHOD

Use "V" rigid plates, microtiter carriers, IEC Head # 976 with UK centrifuge or 2% head with PR-2 or PR-J IEC.

1 x 10^6 or 3 x 10^6 cells may be stained per well (for 1 x 10^6 add 200 λ per well at 5 x 10^6/ml; for 3 x 10^6 add 200λ per well of cells at 1.5 x 10^7/ml.

Balance microtiter plates; centrifuge at 1200 rpm (~ 290g) for 3 min. (Using PR-2 upstairs, set rheostat on 27 when using 2 carriers) timer for 6 minutes

Flick with wrist action to remove supernatant; invert plate and allow to drain on paper towel

Resuspend pellet before adding any liquid. If using microtiter mixer, set on #4 for 3 min. Hand held vibrator may also be used by attaching vibrator to one hand holding plate [redacted] with the other. Cells may also be resuspended in 50 λ liquid by blowing at the pellet in the center of microtiter well above surface of liquid through a Pasteur pipet.

Add 20λ R65 at 1/120 or R anti T @ 1/5; resuspend by above method

Incubate in ice for 20 min.

Resuspend after incubation by above method because cells settle during incubation.

Add 150 λ D's PBS + 5% FCS; resuspend; centrifuge, flick, resuspend pellet.

Use 200λ D's PBS + 5% FCS to do 3 washes

Add 20 λ R-goat anti rabbit at 1/20; incubate for 20 min and follow the above washing procedure.

Pellet, resuspend add 40 λ FCS mix very well and smear for a thin smear
20 λ for a thick smear
or if stained at 1 x 10^6/well add 100 λ D's + 5% FCS to pellet, resuspend well and add to 1.9 ml D's + FCS ;
cells will be at 5 x 10^5 ml, but 0.2 ml in cytocentrifuge will have ~ 10^5/slide cytocentrifuge slide

Microtiter "V" plates - Cooke Engineering obtained through SP.
Microtiter Carriers - " "