Benzidine Staining of Murine CFU-E and BFU-E in Methylcellulose Cultures

Protocol A

Materials

0.2% benzidine dihydrochloride in 0.5 M acetic acid
30% hydrogen peroxide (superoxide)

Procedure

The staining reagent consists of 100 ml of the benzidine solution to which 0.4ml of hydrogen peroxide is added just prior to use. One ml of this reagent is added to each petri dish and 5 min. later, the dish is scored for the numbers (proportion) of colonies which are uniformly benzidine-unreactive (color-less), uniformly benzidine-reactive (blue) and colonies containing both reactive and unreactive cells (mixed colonies containing both differentiated, hemoglobin containing and non-erythroid cells).

Reference: In Vitro Aspects of Erythropoiesis (Ed MJ Murphy Jr) Springer-Verlag. New York 1978: Appendix 11, p 266.

Protocol B

Materials

DAB: Diaminobenzidine Tetrahydrochloride (Sigma Chemicals D5637)
Dissolve 1g of DAB in 20 ml of 0.1 M Phosphate buffer, pH 7. Store aliquots frozen at -20°C.
For staining: prepare just before use:
0.5 ml of DAB dilution
10 ml PBS
5 – 10 µl of 30% Hydrogen Peroxide (H_2O_2)

Procedure

Mix and layer carefully 1 ml on top of the methylcellulose culture and incubate at 37°C for 10 to 15 minutes. Cooling of DAB mixture at 4°C before laying on the methylcellulose may decrease the background staining.

Notes: These protocols have not been tested by StemCell Technologies