untitled protocol

Yuan Yao,morigen

Abstract

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Materials

- 100 mg Hoechst 33258 analog orb363809 by biorbyt
- Tris-HCl (Tris-Hydrochloride), 100gm H5121 by Promega
- Rifampicin RB0808.SIZE.25g by Bio Basic Inc.
- Cephalexin Hydrate, USP Grade C-800 by Gold Biotechnology

Protocol

Step 1.
Cells were grown to OD450=0.15 in ABTGcasa medium at 37°C;

Step 2.
Supplement with rifampicin (300 µg/ml) and cephalexin (10 µg/ml) for three to four generations.

Step 3.
Collect cells by centrifugation at 4 °C;

Step 4.
Resuspend cells in 70% ethanol;

Step 5.
Wash by centrifugation with Tris-HCl buffer (pH 7.5);

Step 6.

Immediately, the Hoechst 33258 was used to stain the cells. Incubate 30 min on ice;

Step 7.
After staining and washing, resuspend the cell pellet in 0.5 ml, according to the configuration of the sample uptake on the cytometer;

Step 8.
Adjust the threshold of Hoechst with 5000;

Step 9.
Low speed, so that cells flow through the cytometer at about 100-1000 cells per second. Slower than 100 cells per second gets very boring. Faster than 1000 cells per second may lead to problems because the computer may not keep up or because two cells close together in the laser beam may be seen by the cytometer as a single "event";

Step 10.
After the flow rate was normal, the data were recorded and 10000 cells were recorded.