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Protocol status: Working

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Flow cytometry

PLOS One

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ATTACHMENTS

[j.1365-2958.2003.03695.x.pdf](#)

MATERIALS

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

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12302

Keywords: ABTGcasa
medium, rifampicin,
cephalexin

- 1 Cells were grown to OD₄₅₀=0.15 in ABTGcasa medium at 37°C;
- 2 Supplement with rifampicin (300 µg/ml) and cephalexin (10 µg/ml) for three to four generations.
- 3 Collect cells by centrifugation at 4 °C;
- 4 Resuspend cells in 70% ethanol;
- 5 Wash by centrifugation with Tris-HCl buffer (pH 7.5);
- 6 Immediately, the Hoechst 33258 was used to stain the cells. Incubate 30 min on ice;
- 7 After staining and washing, resuspend the cell pellet in 0.5 ml, according to the configuration of the sample uptake on the cytometer;

- 8 Adjust the threshold of Hoechst with 5000;
- 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";
- 10 After the flow rate was normal, the data were recorded and 10000 cells were recorded.