Flow Cytometry Sample Preparation

This protocol describes washing and diluting cells from an Erlenmeyer culture for measurement on the flow cytometer.

Materials

- Erlenmeyer culture
- PBS buffer

Equipment

- Microcentrifuge
- 1.5 mL Eppendorf tubes
- Pipettes and pipette tips

Protocol

1. Take 1 mL of the Erlenmeyer culture and spin it down for 2 min at 3000 × g.
2. Discard the supernatant and resuspend the cells in PBS.
3. Spin down again for 2 min at 3000 × g and resuspend in PBS a second time.
4. Dilute the washed suspension 1000× in PBS. The sample is now ready to measure.

Prepare the samples shortly before measuring, and keep them on ice if there is a delay

In Vitro Aptamer Assay

This protocol describes the aptamer binding buffer and the quencher-based fluorescence assay.

Materials

- Aptamer with fluorophore
- Quencher
- HEPES, NaCl, $MgCl_2 \cdot 6H_2O$, KCl
- Milli-Q water

Equipment

- Plate reader with fluorescence channels
- Black 96-well plate
- Heat block or thermocycler (95 °C)
- Pipettes and pipette tips

Binding Buffer, 50 mL

Final composition: 20 mM HEPES, 1 M NaCl, 10 mM $MgCl_2$, 5 mM KCl.

Component	Final	From solid	From 1 M stock
HEPES (MW 238.30)	20 mM	238 mg	1.00 mL
NaCl (MW 58.44)	1 M	2.92 g	50 mL of 1 M
$MgCl_2 \cdot 6H_2O$ (MW 203.30)	10 mM	102 mg	0.50 mL
KCl (MW 74.55)	5 mM	18.6 mg	0.25 mL
Milli-Q water	–	to 50 mL	to 50 mL

Assay

1. Mix the aptamer and the quencher in binding buffer.
2. Heat to 95 °C for 6 min.
3. Cool to room temperature for 30 min, protected from light.
4. Measure in the plate reader at excitation 485 nm and emission 535 nm.

Include a buffer-only blank, an aptamer-only control (maximum signal) and an aptamer with quencher control (background) on every plate