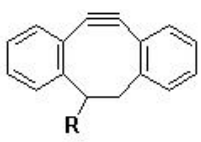
FLOW CYTOMETRY & FLUORESCENCE-ACTIVATED CELL SORTING (FACS) PROTOCOL

Purpose

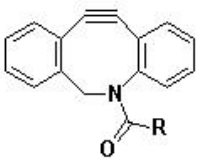
Total time: 120 minutes

Waiting time: 50 minutes

The principle of this technique is the individual quantitative and qualitative identification of particles in a liquid (here bacteria in PBS). The measured signals depend on the particles optical features, they can be inherent like the size (Forward scatter FSC) and the granularity (Side scatte, SSC), or also induced like the signal of a fluorescent molecule.



DIBO



DBCO

Here, the protocol is to check a click chemistry reaction thanks to a fluorescent DIBO molecule. DIBO or dibenzocyclooctyne is a molecule which allows a copper free click chemistry. Thanks to it, this reaction can be done in the bacterial culture medium without copper, toxic for the bacteria. This method also works with DCBO.

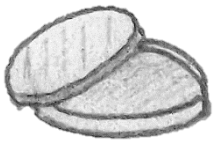
In order to check if the click succeed, a fluorescent DIBO is used.

KEYWORDS: flow-cytometry, click-chemistry, DIBO, FACS.

Resume

Click reaction → Cell fixation → Flow cytometry → Results analysis

Materials



Bacterial culture

Pipettes

PBS

Tubes

- Paraformaldehyde (PFA)
- Fluorescent DIBO (FITC)
- Centrifuge
- Cytometer

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Protocol

Click-reaction with DIBO-FITC

1. Centrifugate the culture at 4 000rpm for 10 minutes.
2. *Remove the supernatant and wash once : take the pellet back into 1 mL of PBS and centrifugate at 4 000rpm for 10 minutes.*
3. Take the bacteria pellet back into the DIBO-PBS solution. 25 µM of DIBO for 50µL at 5.10^9 bacteria/mL.

4. Incubate away from light at room temperature for 5 minutes.
5. Make 3 washes in 1mL of PBS by centrifugating at 10 000g for 3 minutes. Take the bacteria back in 500µL of PBS.

Cell fixation

6. Resume the cell pellet per 500µL of the fixing solution: 3% paraformaldehyde (PFA).
7. Incubate for 15-20 minutes at room temperature.
8. Centrifugate at 500g (1 500rpm) for 10 minutes and repeat with 500µL of PBS.
9. **Flow cytometry**. Run the sample through the cytometer with the adapted settings.

Troubleshooting

Since bacteria are very small organisms, it is difficult to differentiate them from particles already present in PBS or LB.

To solve this problem, a bacterial quantification can be done with a double labelling with a RFP expression in the bacteria in addition of the DIBO fluorescent click reaction.