# Flow cytometry protocol

**Name:**

Flow cytometry to analyze fluorescence levels in single tubes (basics)

**For what:**

To analyze single cell fluorescence levels

**Parent:**

Chemical: Cycloheximide

**Materials:**

A flow analyzer like LSRII Fortessa. Plastic tubes for flow cytometry. 8 peak fluorescence beads for flow cytometry.

**Procedure:**

1. If you want to block translation in your samples, dilute cycloheximide 1:1000 in each sample.

2. Always perform beads measurements BEFORE and AFTER measuring samples, in order to ensure that the LSRII Fortessa worked properly.

3. Transfer 500 µl of your yeast culture in the plastic tube for flow cytometry.

4. If using cycloheximide, wait 30 min before starting measuring at the LSRII Fortessa.

5. Before measuring, make sure that the LSRII Fortessa is switched on and running on high speed for at least 10 min. Make also sure that the LSRII Fortessa's fresh sheath fluid tank is full and the waste tank is empty. Otherwise, substitute the tanks.

6. Start the FACSDiva software and set up your experiment.

7. Check that the correct filters are placed correctly.

8. Before measuring your samples, always make a pre-measurement, where you check if the FSC, SSC, and PMT for fluorescent channels values are good for your experiment.

9. Measure beads.

10. Measure your samples one by one by plugging the plastic tubes to the LSRII Fortessa injector.

11. Measure beads.

12. When you are done, you have to clean the machine. First plug in a tube containing "clean solution" and run for at least 10 min. Second, plug in a tube containing "rinse solution" and run for at least 10 min. 12. Plug in a tube containing water and shut down the machine, OR put it on stand-by.

**Protocol evaluation:**

The FSC-SSC graph tells if the yeast culture were well grown and in exponential phase.

**Notes:**

Always visualize time VS a fluorescent channel in order to make sure that the pressure of the fluidics is constant during your measurement. Always record all three parameters of fluorescent channels: A, H, and W.