FACS PROTOCOL

1. Dilute your probe (e.g. fluorescent antibody, HER-2) in the appropriate amount (here: 1:2000) in 1X PBS
2. Mix 150 µL of each spheroplast sample and each of the non-spheroplast samples (negative control) with 350 µL of 1x PBS containing your probe of choice.
3. Incubate for 1 hour at room temperature under permanent agitation.
4. Centrifuge at 14,000 rpm for 1 min and wash in 1x PBS. Repeat this procedure for two more washing steps.
5. If you used in the previous step direct fluorescent antibody (we used: anti-Affibody Alexa488 antibody), the cells are resuspended after washing in 1 mL of fresh 1X PBS. Then you can continue at step 8 and perform your FACS analysis.

   If the previous probe does not fluoresce, a second incubation is required with a fluorescent antibody is required (e.g. anti-PentaHis Alexa 647 antibody targeting the His-tag of recombinant HER-2). Contiune at step 6
6. Incubate for 1 hour at room temperature under permanent agitation.
7. Centrifuge at 14,000 rpm for 1 min and wash in 1X PBS. Repeat this procedure for two more washing steps.
8. Finally re-suspend your bacterial culture in 1mL of fresh 1X PBS.
9. Analyse the samples in the FACS machine.

Settings used for our FACS analysis:

- FSC: 11 V
- SSC: 993 V
- FL-1 (488nm laser - Filter: 525nm (±20nm)): 354 V
- FL-6 (640nm laser - Filter: 660nm (±20nm)): 772 V