Additional information for Exploration Kit users working with 10 µg Bioportide vials

Dilution series for BPP Bioportides™ for microorganisms with complex membranes and cell walls

Goal: Determine a suitable amount of BPP Bioportide™ for an organism being tested for the first time. The dilution series is particularly useful if the initial experiment yields no colonies or suspected escape colonies.

Procedure:

- Prepare a dilution series of BPP Bioportide™ alone (recommended) or of the preformed Bioportide–nucleic acid complex. Diluting Bioportide alone lets you test different nucleic acids with the same series.
- We will explore using the BPP Bioportide™ dilution series without nucleic acids.
- Your vial with freeze-dried BPP Bioportide™ **contains 10 µg of protein**.
- **Dissolve** the lyophilized BPP Bioportide™ **in 10 µl of Buffer solution** (50% glycerol) in a sterile environment. Leave for **20 minutes at room temperature between 17 °C and 25 °C** without agitation. If residual material adheres to the vial wall, pipette gently to dissolve it. This yields a BPP Bioportide™ stock solution of 1,000 ng/µl.
- Prepare **three vials with 9 µl of Buffer solution** and label them **from 1:10 to 1:1,000**. Add 1 µl of the stock to 9 µl of Buffer solution and mix (1:10; 100 ng/µl). Transfer 1 µl of this dilution to the next 9 µl buffer and mix (1:100; 10 ng/µl). Repeat once more (1:1,000; 1 ng/µl).
- Prepare a corresponding dilution series of your preferred nucleic acid. To keep the ratio (w/w) between BPP Bioportide™ and nucleic acid **at 10:1**, start with **100 ng/µl** nucleic acid solution**.** Label three tubes 10 ng/µl, 1 ng/µl and 100 pg/µl, and add 9 µl of nuclease-free diluent to each. Add 1 µl of the 100 ng/µl solution to the first tube and mix (10 ng/µl). Transfer 1 µl from the first tube to the second and mix (1 ng/µl). Repeat once more (100 pg/µl).
- Preparing BPP Bioportide™-nucleic acid complexes. Label 4 tubes as shown below. Add 3 µl of a buffer suitable for cell contact to each tube. Then add 1 µl of the indicated BPP Bioportide™ dilution and 1 µl of the corresponding nucleic acid dilution.

Tube	BPP Bioportide™	Nucleic acid
Stock	1,000 ng/µl	100 ng/µl
1:10	100 ng/µl	10 ng/µl
1:100	10 ng/µl	1 ng/µl
1:1,000	1 ng/µl	100 pg/µl

- Incubate for 10 min at room temperature. Add cells and continue with the protocol for your organism.