BPP Bioportide™ Fungal Transformation Kit for Ascomycota

Cat. no. BPP-BPort-003

Product Overview

The BPP Bioportide™ kit provides a ready-to-use system for efficient transformation of various microorganisms, including *Sordaria* and other ascomycetes. The kit contains **lyophilized BPP Bioportide™**, and a separate vial of **sterile 50% glycerol** for storage at -20°C. BPP Bioportide™ facilitates the uptake of nucleic acids without preparing competent cells, heat shock, electroporation or conjugation.

Components Supplied

1. **BPP Bioportide™**: Contains HEPES and arginine as buffer material. One vial contains **10 µg** of lyophilized BPP Bioportide™ which is sufficient for at least 10 transformation reactions.
2. **BPP Bioportide™ Buffer**: delivered in a separate vial that contains 500 µl of sterile 50% glycerol.

Storage, Handling and Stability

- Store lyophilized BPP Bioportides™ at **-20°C** or -80°C for long-term storage.
- The **BPP Bioportide™ buffer** can also be stored at **-20°C** or -80°C.
- Once dissolved, the **BPP Bioportide™ working solution** must be stored at **-20°C**.

Caution

BPP Bioportides™ should be handled with care. The effects of prolonged or unprotected exposure are unknown. Users should always follow mandatory lab safety regulations and especially adhere to the following safety standards:

- Avoid skin or eye contact. Wearing gloves and eye protection when handling the product is strongly recommended. In case of contact, rinse skin or eyes thoroughly with water immediately.
- Do not inhale or consume. Avoid the formation of and exposure to aerosols, e.g. by working under a safety cabinet or in well-ventilated workspaces. Avoid ingestion.
- Do not use it while pregnant or breastfeeding.
- Use only as described in this manual. Do not leave open on a bench.

Product Description

BPP Bioportide™ is a recombinant protein with a molecular weight of roughly **37.5 kDa**. The exact size depends on the variant of BPP Bioportide™. Variants differ in their cell-penetrating domains, which are designed for specific cell envelope compositions. The protein facilitates the transport of nucleic acids, ranging from **22 nt to >10 kb**, into cells of a wide range of organisms, i.e. bacteria, archaea, fungi, plants, and animals (depending on the BPP Bioportide™ in use). Both **RNA** and **DNA**, including single- and double-stranded, linear or circular forms, can be transferred reliably. BPP Bioportides™ are designed for robust performance in extreme environments (up to 80°C, pH > 9.0, high salt concentrations).

Key Features

- No need for competent cells, heat shock, electroporation or conjugation.
- Works with **nanogram to microgram** quantities of nucleic acids in **Sordaria**.
- Suitable for DNA and RNA, linear and circular, single- and double-stranded, short and long (≥10 kb).
- The quantity ratio between **BPP Bioportide™** and **nucleic acid** should be **~ 10 : 1**. For further details, especially if you wish to introduce larger nucleic acids into your cells, please refer to document "Ratio calculation" on our website.
- Short and easy protocol saves a lot of time and results in low total cost of experiment.

General Considerations

- Adapt our standard protocol as a starting point to the specific needs of your strain. If you need further guidance, please do not hesitate to contact us.
- BPP Bioportide™ transformation works best with early to mid-log phase cells. For bacterial transformation the optimal OD_{600} is 0.4 – 0.6. For Sordaria protoplast transformation grow the strain for 2-3 days (approximately 6-8 inoculation pieces).
- As the BPP Bioportide™ carrier domain has an affinity for polysaccharides, avoid those within the medium that you use for incubation.
- Consider using a known and easy to handle plasmid as control in addition to your main experiment.
- Consider carrying out the transformation without any pre-treatment – using for example, Vinotaste Pro or Chitinase – as a control experiment.

General Transformation Protocol for *Ascomycetes*, in particular for Sordaria Protoplasts

1. **Material preparation**
    - Prepare Protoplasts / your strain **according to your standard protocol**.

      For **Sordaria protoplast** transformation grow the strain in Fernbach flasks with 100-150 ml CM medium for 2-3 days (approximately 6-8 inoculation pieces).
      After Protoplast preparation, count the protoplasts in a Thoma chamber under a microscope, centrifuge at 3500 rpm for 4 min, discard supernatant and dissolve pellet in the required amount of transformation buffer TPS (2×10^8 / ml). Vortex until the pellet is dissolved and keep on ice. (*TPS: 1 M Sorbit, 80 mM $CaCl_2$, pH 7.5.)*
    - At the end, one reaction of BPP Bioportides™ corresponds to a volume of transformed cells of 1 ml, which is available for plating. Please calculate the material for your planned experiments accordingly.
    - Prepare **CM (Complete medium), CMS agar plates (CM with 0.8 M Sucrose, solid media with 2% Agar) and Top-Agar** with appropriate antibiotics for selection.
2. **Prepare BPP Bioportide™ Working Solution**
    - Add **10 µl of BPP Bioportide™ buffer** to one vial of BPP Bioportide™ (yielding a **1 µg/µl working solution**). Mix gently by pipetting.
    - Incubate at **room temperature** for **20 minutes** without agitating.
    - **For Sordaria Protoplast transformation**: Prepare a **1:10 dilution** (**0.1 µg/µl**).
      4.5 µl is required for one reaction.
    - If you wish to transform other strains that have not yet been tested using BPP Bioportide™ transformation, please dilute only part of the working solution; you can easily store it at -20 °C and use it again later. To determine the optimum, non-toxic concentration of BPP Bioportide™, it is recommended to carry out a series of dilution experiments, as described in document "Dilution-series-Exploration-Kits".
3. **Prepare Nucleic Acid Mixture**
    - Mix **4.5 µl** of **0.1 µg/µl BPP Bioportide™ Working solution** with **1 µl** of nucleic acid (**1.5 µg**). Prepare your nucleic acid accordingly.
    - Mix gently by pipetting. Incubate the mixture at **room temperature** for **10 minutes**.
4. **Transform the Cells**
    - Add **100 µl of protoplasts / cells** to the BPP Bioportide™/nucleic acid mixture (step 3) and keep at **RT** for **30 minutes without shaking**.

5. **Recover and Plate**
    - Add **900 µl of TPS buffer** to the cells and plate 4 x 250 µl on CMS plates.
    - Incubate plates at least **2 hours - overnight at 27 °C**.
    - Layer with 9 ml Top-Agar; add antibiotics for selection.

Documentation and support

Customer and technical support

Visit www.bpp-peptides.com for the latest service and support information.

Limited product warranty

Better Peptides Platform GmbH warrants its products as set forth in the General Terms and Conditions of Sale at www.bpp-peptides.com. If you have any questions, please contact Better Peptides Platform GmbH at www.bpp-peptides.com.

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Revision

The information in this user guide is subject to change without notice.

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