BADISCHE PEPTIDE & PROTEINE

BPP Bioportide™ 10x Exploration Kits for Transformation / Transfection

Product Overview

The BPP Bioportide™ Exploration Kits provide a novel ready-to-use system for efficient transformation / transfection of various microorganisms and other cell types.

Each Exploration Kit contains 10 different BPP Bioportides™, numbered from one to ten, with number one being supposed to be the most suitable for the group of organisms a certain kit is dedicated for.

Note: Due to the complete novelty of this innovative reagent and the multitude of organisms to be considered there is only limited data available. However, as the demand from researchers working with organisms that are difficult or impossible to transform is very high, test products are made available for purchase.

Positive results are already existent for *E. coli*, for several strains of *cyanobacteria*, e.g. *Synechocystis* and *Nostoc*, and for tobacco plants. Several other organisms and cell types are currently under investigation. The suggested protocols in this manual have been developed based on the respective design of a certain BPP Bioportide™ and literature data and should be understood as a starting point.

Product Description

BPP Bioportide™ is a recombinant protein with a molecular weight of roughly **37.5 kDa**. The exact size depends on the kind of BPP Bioportide™. They are distinguished by their cell-penetrating domains, which were designed for specific cell envelope compositions. The second domain binds nucleic acids non-specifically and reversibly. The protein enables 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.
In all organisms tested so far, **no pre-treatment** causing cell stress, such as heat shock, electroporation, or any other kind of competency, was necessary for successful transformation.
BPP Bioportides™ are designed for robust performance in extreme environments (up to 80°C, pH > 9.0, high salt concentrations).

Components Supplied

1. **BPP Bioportide™**: Each Kit contains 10 vials with different **BPP Bioportides™**. Each vial contains **10 µg** of a specific **lyophilized BPP Bioportide™** which is equal to **ten reactions** for transformation or transfection. One reaction corresponds to a volume of transformed cells of 1 ml, which is available for plating. Contains HEPES and arginine as buffer material.

Article number	Articel name	number reactions
BPort-S10F	BPP Bioportide™ 10x Exploration Kit for Fungi	100
BPort-S10P	BPP Bioportide™ 10x Exploration Kit for Plants	100
BPort-S10GNB	BPP Bioportide™ 10x Exploration Kit for gram-negative bacteria	100
BPort-S10GPB	BPP Bioportide™ 10x Exploration Kit for gram-positive bacteria	100

2. **BPP Bioportide™ Buffer**: The fluid component is 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, respectively.
- 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. As effects to prolonged or unprotected exposure are not foreseeable, precautions are strictly advised, and 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.
- Only use within the parameters detailed in this manual and do not leave open on a bench.

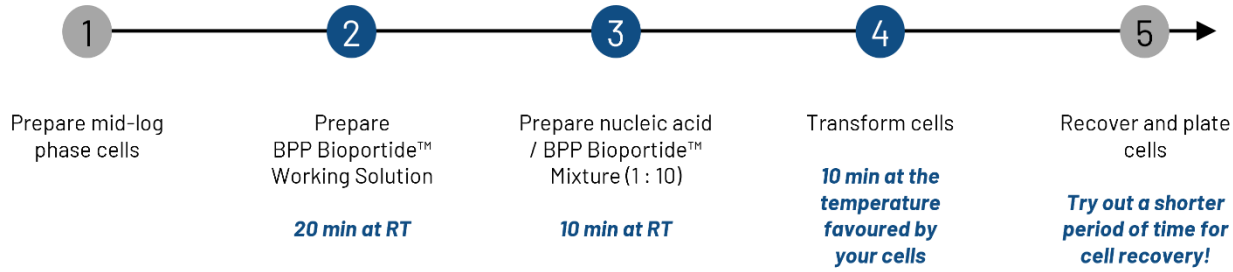
Key Features

- No need for competent cells, heat shock, or electroporation.
- Efficient transformation, with up to **3 × 10^8 cfu/µg** for **pUC19** in ***E. coli***, and **6 × 10^5 cfu** for a **10 kb plasmid** in *E. coli*. Efficient transformation, with up to ~300 CFU for *Synechocystis* transformed with 10 ng of a 6 kbp linear integrative plasmid (DNA).
- Works with **picogram** quantities of nucleic acids in ***E. coli.*** Works with **nanogram** quantities of nucleic acids in ***cyanobacteria*** like ***Synechocystis or Nostoc.***
- Short / long linear, circular single / double stranded DNA / RNA, large (~10 kb) plasmids.
- Short and simple protocol saves a lot of time.
- Low total cost of experiment
- First-to in new organisms.

General Considerations

- Adapt our standard protocol as a starting point to the specific needs of your strain. If the BPP Bioportide™ protocol does not offer enough details, proceed according to your standard protocol, as this contains the optimum conditions for your strain. If you are not sure, 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.
- As the carrier domain has an affinity for polysaccharides, avoid those within the medium that you use for incubation to bring in contact your nucleic acid / BPP Bioportide™ complex with your cells.
- Consider using a known and easy to handle (methylation adapted) plasmid as control in addition to your main experiment.
- The quantity ratio between **BPP Bioportide™** and **nucleic acid** should be **10 : 1**.
- The volume during contact should be as low as possible to avoid dilution of cells and the BPP Bioportide™/nucleic acid complex and enhance contact events.
- The cationic residues of the cell penetrating moiety of the BPP Bioportide™ will interact with negatively charged domains within the cell walls and the membrane. In gram-positive bacteria the formation of pores will be enhanced by high curvature and in fungi endocytosis of the BPP Bioportide™ / nucleic acid complexes will be more easily mediated.

General Transformation Protocol – WORKFLOW



1. **Material preparation**
   - Grow mid-log phase cells according to your standard protocol. Optimal OD_{600} for BPP Bioportide™ transformation in bacteria would be 0.4. Centrifuge cells at low velocities and resuspend in a small volume of appropriate medium or transformation buffer, e.g. 100 µl to 2 ml. By using BPP Bioportide™ for transformation fewer cells are needed than usual.
   - 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 the appropriate **medium** and **agar plates** with and without appropriate antibiotics.
2. **Prepare BPP Bioportide™ Working Solution**
   - Add **10 µl of BPP Bioportide™ buffer** to one vial of BPP Bioportide™ (yielding a **1000 ng/µl working solution** sufficient for 10 reactions).
   - Incubate at **room temperature** for **20 minutes** without agitating.
3. **Prepare Nucleic Acid Mixture**
   - Mix **1 µl** of nucleic acid (**100 ng/µl**) with **1 µl** of **BPP Bioportide™ Working solution** (sufficient for one reaction).
   - Incubate the mixture at **room temperature** for **10 minutes**.
4. **Transform the Cells**
   - Add **50 µl of cells** (see step 1) to the BPP Bioportide™/nucleic acid mixture (**2 µl**; see step 3)
   - Keep the transformation preparation for **10 minutes** at a temperature that is suitable for your cells (e.g. according to your standard transformation protocol).

5. **Recover and Plate**
    - Add **950 µl of appropriate medium** to the cells and incubate according to your standard transformation protocol regarding temperature, duration for recovery and agitation. We also recommend testing a shorter incubation period, as the BPP Bioportides™ are less harmful to the cells than the standard methods.
    - Plate and incubate the transformed cells according to your standard procedures.

Exemplary Protocols

Fungal transformation

Note: For fungi that were previously difficult to transform, enzymatic pretreatment of the cell wall prior to BPP Bioportide™ transformation should be carried out as an additional experiment.

- A cell aliquot is grown in 5 ml YPAD medium at **30°C** and **200 rpm** overnight.
- Dilute overnight culture to 10^6 in the same medium.
- **2.5 x 10^8 cells** are transferred to **50 ml** of YPAD medium to arrive at a concentration of **5 x 10^6 cells/ml**.
- Cultivate at **30°C** and **200 rpm** for up to four hours to arrive at a cell density of **2 x 10^7 cells/ml**. The cells are harvested, and the cell pellet is washed in sterile water twice. The pellet is then thoroughly resuspended in 1 ml sterile water. **100 µl of the suspension contain 10^8 cells**.
- An aliquot of 100 µl is used for a control transformation experiment if desired and another aliquot of 100 µl is used for BPP Bioportide™ transformation.
- The control cells are resuspended in a transformation mix of **240 µl PEG3355 (50% w/v) and 36 µl 1M Lithium acetate**. Plasmid DNA and single stranded DNA have a concentration of 2 mg/ml and are added to the transformation mix topping it to 360 µl.
- The cells for BPP Bioportide™ transformation are resuspended in 500 µl of YPAD medium. Add a BPP Bioportide™ / nucleic acid complex with the desired amount of nucleic acid to this suspension. BPP Bioportide™ and nucleic acid complex are prepared as described under General Transformation Protocol.
- The **control cells** are incubated at **42°C for 40 minutes** and the **BPP Bioportide™ treated cells** are incubated **at 30°C and 200 rpm for one to two hours** before they are streaked out on solid plates.

Protocol based on:

[1] Transformation of *Saccharomyces cerevisiae* and other fungi - Methods and possible underlying mechanism, Kawai et al., Bioengieered Bugs 1:6, 2010

[2] Yeast cell morphology and sexual reproduction – A short overview and some considerations, Knop, C.R. Biologies, 334, 2011

Gram-Positive Bacteria

- Electroporation can be used as a control method. For BPP Bioportide™ mediated transformation, prepare a fresh culture in **10 ml BHI** medium for **2 hours** at **37°C** and **300 rpm**.
- At **OD_{600} of <0.4** the cells can be brought into contact with the complex of BPP Bioportides™ and nucleic acids. The BPP Bioportide™ and nucleic acid complex is added to 1 ml of the cells and this mixture is again incubated at **37°C** for one to **two hours**.
- 200 µl of the culture are streaked out on a **CV-blood agar plate**.

Protocol based on:

[1] Characterization of *Enterococcus faecalis* in different culture conditions, Kim et al., Nature Scientific Reports, 2020

Archaea

Note: For archaea that were previously difficult to transform, pretreatment of the cell wall prior to BPP Bioportide™ transformation should be carried out according to standard protocols as an additional experiment.

- For **methanococcus** and similar organisms, grow cultures in **Whitman medium**.
- This part of the protocol is dedicated to methanococcus and other euryarcheotae. You need to freshly grow out your archaea in defined Whitman medium.
- The cells need to be at an OD_{600} of between 0.2 and 0.4. Centrifuge them at low velocities. Replace the supernatant with a transformation buffer of 50 mM Tris pH 7.5, 0.1 M sucrose, 0.1 M NaCl and 10% PEG8000. PEG8000 is optional.
- The complex of BPP Bioportide™ and nucleic acid is prepared as described above. The cells are resuspended in the transformation buffer and the BPP Bioportide™ / nucleic acid complex is added. The suspension is now incubated at 37°C without agitation for one hour.

- Then, the cells are removed from the transformation buffer and resuspended in Whitman medium. Run the culture in a way that is viable for the comforting of your specific strain and streak the cells on plates after preparation of a dilution series.

Protocol based on:

[1] Genetic Transformation in the Methanogen *Methanococcus voltae* PS, Bertani et al., Journal of Bacteriology, 1987

[2] High-efficiency transformation of archaea by direct PCR products with its application to directed evolution of a thermostable enzyme, Song et al., Microbial Biotechnology, 2020

Documentation and support

Customer and technical support

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Manufacturer

Badische Peptide und Proteine GmbH, Waldshuter Straße 5, 68239 Mannheim, Germany

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Revision

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