# BPP Bioportide™ 10x Exploration Kits for Transformation / Transfection

# Cat. no. BPP-BPort-S10A / BPP-BPort-S10F / BPP-BPort-S10GPB / BPP-BPort-S10GNB

## 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 Bioportide™ variants, numbered from one to ten. Variant 1 is expected to be the most suitable for the group of organisms a certain kit is dedicated for.

**Note:** Data are limited for many target organisms. If you have questions, please contact us. We are happy to advise based on our experience with different organisms and will treat your project details confidentially.

**Positive results** have been obtained for *E. coli*, several strains of *cyanobacteria*, e.g. *Synechocystis*, *Synechococcus*, hydrogen-oxidizing bacteria, *Sordaria*, and tobacco plants. Several other organisms and cell types are currently under investigation.

**The suggested protocols in this manual have been developed based on the BPP Bioportide™ design and available 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 variant of BPP Bioportide™. Variants differ in their cell-penetrating domains, which are designed for specific cell envelope compositions. The second domain binds nucleic acids non-specifically and reversibly. 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.
In all organisms tested so far, **no pre-treatment** causing cell stress, such as heat shock, electroporation, or other method of preparing competent cells, 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 sufficient for at least 10 transformation or transfection **reactions.** Contains HEPES and arginine as buffer material.

2. **BPP Bioportide™ Buffer**: delivered in a separate vial that contains 500 µl of sterile 50% glycerol.

| Article number | Article name | number reactions |
|---|---|---|
| **BPort-S10F** | BPP Bioportide™ Exploration Kit for Fungi | 100 |
| **BPort-S10GNB** | BPP Bioportide™ Exploration Kit for gram-negative bacteria | 100 |
| **BPort-S10GPB** | BPP Bioportide™ Exploration Kit for gram-positive bacteria | 100 |
| **BPort-S10A** | BPP Bioportide™ Exploration Kit for Archaea | 100 |

## 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.

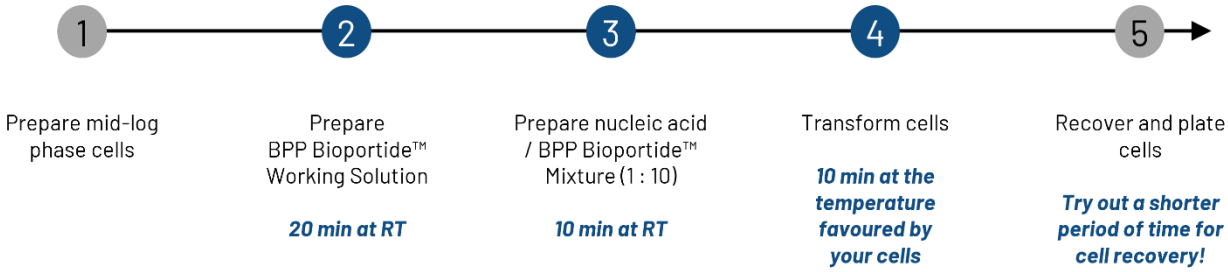
## Key Features

- No need for competent cells, heat shock, electroporation, or conjugation.
- Data on **transformation efficiency** in ***E. coli*** and **cyanobacteria** can be found in the corresponding manual and on our website. Please also refer to our publication on cyanobacterial transformation (https://doi.org/10.3389/fpls.2026.1812316).
- Works with **picogram** quantities of nucleic acids in ***E. coli.*** Works with **nanogram** quantities of nucleic acids in ***cyanobacteria*** like ***Synechocystis or Synechococcus.***
- Suitable for DNA and RNA, linear and circular, single- and double-stranded, short and long, (>10 kb).
- Short and simple 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.
- 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 (methylation adapted) plasmid as control in addition to your main experiment.
- The general protocol below uses a 10:1 BPP Bioportide™-to-nucleic-acid mass ratio as a starting condition.
- For a new organism, first try the general protocol below with a 10:1 BPP Bioportide™-to-nucleic-acid mass ratio. If this does not give the expected result, or if you use short RNA (20–300 nt) or a large plasmid (8–25 kb), use our Molar Ratio Calculator to plan additional conditions.
- 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.

## 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 – 0.6. 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). Mix gently by pipetting.
   - 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). Mix gently by pipetting.
   - Incubate the mixture at **room temperature** for **10 minutes** without agitating.
4. **Transform the Cells**
   - Add **50 µl of cells** (see step 1) to the BPP Bioportide™/nucleic acid mixture (**2 µl**; see step 3). Mix gently by pipetting, if suitable for your cells.

- Keep the transformation preparation for **10 minutes** (without agitating) 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.

- Grow a 5 ml culture in YPAD medium at **30 °C** and **200 rpm** overnight. Determine the cell density of the overnight culture.
- Inoculate **50 ml** of YPAD medium with **2.5 x $10^8$ cells** 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**.
- Harvest the cells, wash the cell pellet twice in sterile water and thoroughly resuspend it in 1 ml sterile water. Each **100 µl aliquot of the suspension contains approximately $10^8$ cells**.
- Prepare two 100 µl aliquots: one for LiAc/PEG control transformation and one for BPP Bioportide™ transformation.

Control – LiAc/PEG

- Centrifuge one 100 µl aliquot and resuspend it in 360 µl of transformation mix, consisting of **240 µl PEG3350 (50% w/v), 36 µl 1M Lithium acetate**, 100 ng plasmid DNA plus water to 34 µl and 50 µl single stranded DNA (2 mg/ml).
- Incubate at **42 °C for 40 minutes.** Plate onto SC selection medium.

BPP Bioportide™ transformation

- Prepare BPP Bioportide™ - nucleic acid complex as described under General Transformation Protocol.
- Centrifuge one 100 µl aliquot and resuspend the cells in 500 µl YPAD medium. Add 2 µl BPP Bioportide™ / nucleic acid complex to this suspension.
- Incubate the **BPP Bioportide™ treated cells** at 30 °C and 200 rpm for 1-2 h. Plate on SC selection medium.

**Protocol based on:**

[1] Transformation of *Saccharomyces cerevisiae* and other fungi - Methods and possible underlying mechanism, Kawai et al., Bioeng Bugs. 2010 Nov-Dec;1(6):395–403. doi: 10.4161/bbug.1.6.13257

[2] Yeast cell morphology and sexual reproduction – A short overview and some considerations, Knop, M.; C.R. Biologies 334 (2011) 599-606. doi: 10.1016/j.crvi.2011.05.007

## Gram-Positive Bacteria

- Electroporation can be used as a control method. Follow the standard electroporation protocol for your strain.
- If your strain prefers different growth media, please feel free to replace the ones mentioned here. Just make sure it doesn't contain any polysaccharides.
- For BPP Bioportide™ mediated transformation, grow a fresh culture in **10 ml BHI** medium for **two hours** at **37 °C** and **300 rpm**.
- At **$OD_{600}$ <0.4** the cells can be brought into contact with the complex of BPP Bioportides™ - nucleic acids.
- Prepare BPP Bioportide™ - nucleic acid complex as described under General Transformation Protocol.
- Add 2 µl BPP Bioportide™ - nucleic acid complex to 1 ml of the cells. Incubate at **37 °C** for 1-2 h.
- Spread 200 µl of the culture on a **CV-blood agar plate**.
- Incubate at37 °C for 16-24 h, depending on your strain.

**Protocol based on:**

[1] Characterization of *Enterococcus faecalis* in different culture conditions, Kim et al., Sci Rep. 2020 Dec 14;10:21867. doi: 10.1038/s41598-020-78998-5

## 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.

- This part of the protocol is dedicated to **Methanococcus** and other **euryarchaeota**.
- Grow a fresh archaea culture in defined **Whitman** medium to $OD_{600}$ 0.2-0.4.
- Centrifuge the cells (e.g. 6500 x g, 5 min, depending on your strain) and resuspend in 200 µl of transformation buffer: 50 mM Tris pH 7.5, 0.1 M sucrose, 0.1 M NaCl and optionally 10% PEG8000.
- Prepare BPP Bioportide™ - nucleic acid complex as described under General Transformation Protocol.
- Add 2 µl BPP Bioportide™ - nucleic acid complex to the cell suspension and incubate at 37 °C without agitation for 1 h.
- Replace the transformation buffer with Whitman medium. Incubate under the standard growth conditions for your strain. Prepare dilutions and plate on agar.

**Protocol based on:**

[1] Genetic transformation in the Methanogen *Methanococcus voltae* PS, Bertani et al., J. Bacteriol. 1987 Jun;169(6):2730-8. doi: 10.1128/jb.169.6.2730-2738.1987

[2] High-efficiency transformation of archaea by direct PCR products with its application to directed evolution of a thermostable enzyme, Song et al., Microb Biotechnol. 2021 Mar;14(2):453-464. doi: 10.1111/1751-7915.13613

Do you want to transfect **plant** or **mammal cells** using BPP Bioportides™?

Please contact us via service@bpp-peptides.com.

## Documentation and support

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### Revision

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

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