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BPP Bioportide™ Transformation Kit for Microorganisms

Product Overview

The BPP Bioportide™ kits provide a ready-to-use system for efficient transformation of various microorganisms, including *E. coli* and gram-negative bacteria. Each vial contains **lyophilized BPP Bioportide™**, supplemented by a separate vial with **sterile 50% glycerol** for storage at -20°C. This innovative reagent facilitates the uptake of nucleic acids without requiring inducing cell stress, i.e. heat shock, or competent cells, i.e. by means of electroporation or chemical treatment of cell wall and cell membrane.

Components Supplied

1. **BPP Bioportide™**: Contains HEPES and arginine as buffer material. 1 µg of lyophilized BPP Bioportide™ equal to ten reactions for transformation of *E. coli* and *cyanobacteria*. 10 µg of lyophilized BPP Bioportide™ equal to ten reactions for TEST Kits for all other organisms.
2. **BPP Bioportide™ Buffer**: The fluid component is delivered in a separate vial that contains 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, precaution is strictly advised, and users should follow mandatory lab safety regulations at all times and especially adhere to the following safety standards:

- Avoid skin or eye contact. Wearing gloves and eye protection when handling the product is strongly advised. In case of contact, rinse skin or eyes thoroughly with water immediately.
- Do not inhale or consume. Avoid formation of and exposure to aerosols, e.g. by working under a fume hood or in good ventilated workspaces. Avoid ingestion.
- Do not use while pregnant or breastfeeding.
- Only use within the parameters detailed in this manual and do not leave open on a bench.

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Product Description

BPP Bioportide™ is a recombinant protein, with a molecular weight of roughly **37.5 kDa**, in the case of BP17. The exact size depends on the kind of BPP Bioportide™. It is designed for **robust performance in extreme environments (up to 80°C, pH > 9.0, high salt concentrations)**. 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.

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**.
- Works with **picogram** quantities of nucleic acids in ***E. coli***.
- Suitable for gram-negative bacteria; other microorganisms are under investigation.

General Transformation Protocol for *E. coli* and *cyanobacteria*

1. **Prepare the Materials**
   - Prepare SOC medium and **LB agar plates** with appropriate antibiotics.
   - Bring all materials to **37°C**.
2. **Prepare BPP Bioportide™ Working Solution**
   - Add **10 µl of BPP Bioportide™ buffer** to one vial of BPP Bioportide™ (yielding a **100 ng/µl** working solution).
   - Incubate at **room temperature** for **10-20 minutes**.
3. **Prepare Nucleic Acid Mixture**
   - Mix **1 µl** of nucleic acid (100 ng/µl) with **1 µl** of BPP Bioportide™ Working solution.
   - Incubate the mixture at **20-25°C** for **5-10 minutes**.

4. **Transform the Cells**
    - Thaw **50 µl of *E. coli*** cells.
    - Add **2 µl** of the BPP Bioportide™/nucleic acid mixture to the cells and keep at **0-4°C** for **5-10 minutes**.
5. **Recover and Plate**
    - Add **950 µl of SOC medium** to the cells and incubate at **37°C** with shaking (250-450 rpm) for **30-60 minutes**.
    - Perform a dilution series and spread **100 µl** on selection plates.
    - Incubate plates **overnight at 37°C**.

Quality Control

During transformation, it's important to verify the viability of your cells. Spread a portion of your cells on plates without antibiotics to assess the impact of BPP Bioportide™ on cell vitality. Higher concentrations of BPP Bioportide™ may negatively impact cell health over time.

Troubleshooting (*E. coli*)

- **Contaminants**: BPP Bioportides™ are expressed with **kanamycin resistance**. If contamination is a concern, spread the transformed cells on kanamycin plates to verify the plasmid.
- **Stress on cells**: BPP Bioportides™ can stress cells as they pass through bacterial membranes. It's advisable to limit dilution of transformation aliquots beyond **10^{-4}**.
- **Escape CFUs**: Sometimes smaller colonies ("escape CFUs") may not stably integrate plasmids. To check for stable transformation, grow colonies in selective media and perform plasmid prep followed by **restriction mapping** or **site-directed PCR**.

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Supplementary Protocols

Mammalian Cells

1. Add **10 µl of BPP Bioportide™ buffer** to the protein vial.
2. Incubate for **20 minutes** at room temperature.
3. Combine **100 ng nucleic acid** with **1 µg of BPP Bioportide™**.
4. Incubate for **5 minutes** before applying to cells.

Fungal transformation

- 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 for the other protocols.
- 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.

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**.

Archaea

- 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.

Considerations for working with cell walls

- You can adapt our standard protocol as a starting point to the specific needs of your organism.
- Note, that you do not have to prepare competent cells although you can still use them for BPP Bioportide™ transformation. BPP Bioportide™ transformation works best with early to mid log phase cells. 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.
- The volume during contact should be as low as possible to avoid dilution of cells and 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.

Data of Quality Assessment

1. Transformation of **pUC19** in ***E. coli***
2. Transformation of **10 kb BPP-plasmid** in ***E. coli***
3. BPP Bioportide™ identity verified by **polyacrylamide gel electrophoresis**

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You can test the performance of your BPP Bioportides™ to answer two questions:

1. Is the freshly prepared BPP Bioportide™ corrupted?
2. Is the BPP Bioportide stock that has been prepared a few days ago still usable?

Protocol for performance testing

- A 10ng/µl stock of one of your standard plasmids with known transformation efficiency is prepared.
- 1 x LB 2% agar plates with selection antibiotic and without your selection antibiotic need to be prepared.
- The selection plates are used to calculate and compare the **transformation efficiencies (cfu/µg DNA)** between BPP Bioportide™ and heat shock transformed cells.
- The non selection agar plates are needed to calculate **the ratio between** the cfu of the **selection** plate **divided by** the cfu of the **corresponding non selection plate**. This calculates the **transformation frequency**.
- You will need to prepare *E. coli* BL21 cells for your control experiments. All cell samples should have a volume of 50 µl. Prepare calcium competent cells for heat shock and reference experiments. You will also need to prepare log phase cells that you can either use immediately or freeze and use whenever necessary. For the sake of reproducibility, the latter is recommended.
- The complex of BPP Bioportide™ and plasmid DNA is going to be prepared according to the manual. 1 µl of 10 ng/µl DNA is mixed with 1 µl 100 ng/µl BPP Bioportide™.
- Let the complex of BPP Bioportide™ and DNA incubate at room temperature (T=24°C) for 5 minutes. In the meantime, thaw the cells. You will need two samples of log phase cells and two samples of competent cells. Each kind of cells will be transformed using both methods, BPP Bioportides™ and heat shock.
- Use 1 µl of 10 ng/µl DNA on one of the log phase and one of the calcium competent samples. After a lag time of 10 minutes at 4°C, both samples are heat shocked for 30 seconds at 42°C and relaxed on ice or at 4°C for 5 minutes. Simultaneously, the remaining cell samples will be treated with 2 µl of the BPP Bioportide™ and DNA complex. These cells are placed at 4°C for 10 minutes and then immediately treated with 950 µl of SOC medium. The heat shocked cells are treated with SOC after the cells have been relaxed after the heat shock.
- The cells are then incubated for one hour at 37°C while being rotated at 100 rpm, minimally. After that, streak out 100 µl of each sample on a selection and a non selection plate. You can also streak dilution series of the samples and produce technical replicate plates.

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Documentation and support

Customer and technical support

Visit www.badische-peptide-proteine.de for the latest service and support information.

- Contact telephone numbers
- Product support information
- Product documentation
    - User guides, manuals, and protocols
    - Certificates of Analysis
    - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Badische Peptide und Proteine GmbH warrant their products as set forth in the General Terms and Conditions of Sale at www.badische-peptide-proteine.de. If you have any questions, please contact Badische Peptide und Proteine GmbH at www.badische-peptide-proteine.de.

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