BPP Bioportide™ to nucleic acid Ratio Calculation

When you receive your BPP Bioportide™, you will receive your reagent as a freeze-dried powder that contains either 1 µg or 10 µg of the reagent. The manual asks you to dissolve the powder and gives you the resulting concentration as a weight-to-volume concentration. We at BPP found it straightforward to work with weight-to-weight ratios of BPP Bioportide™ to (ribo)nucleic acid. This made sense so long as the DNA we worked with was in the same size range. If you want to work with a larger bandwidth of DNAs and RNAs, it makes sense to compare molar ratios as you need an excess of BPP Bioportide™ without reaching the threshold of BPP Bioportide™ cytotoxicity for your target organism.

Your molar excess of BPP Bioportide™ to (ribo)nucleic acid should be 3 to 10 for small RNAs with a size of 20 to 300 nucleotides and up to 500 for mRNAs with a size of around 1500 nucleotides. For small plasmids of between 2 kb and 5 kb in size, molar ratios may be between 500 and 1000. Larger plasmids of between 8 kb and 25 kb need to be associated with ratios between 1000 and 3000.

In order to make an estimate of the ratio, we assume that the nucleotides are distributed equally. We need to know the size of construct and if the molecule is single stranded or double stranded. The molar mass of the (ribo)nucleic acid is

$$Mol.Weight_{(ribo)nucl.}[\frac{g}{mol}] = 2 \cdot \left(\frac{1000 \cdot x[kb]}{4} \cdot (347.2\frac{g}{mol}A + 322.2\frac{g}{mol}T + 363.22\frac{g}{mol}G + 323.2\frac{g}{mol}C)\right).$$

In the case of RNA, T is to be replaced with U (=324.18 g/mol). The formula applies to double-stranded molecules; for single-stranded molecules, omit the factor 2.

The average BPP Bioportide™ has a molecular weight of 37500 g/mol. The weight of your BPP Bioportide™ stock will be 1 µg or 10 µg per vial. You will usually be asked to prepare a solution with a concentration of 100 ng/µl.

Nucleotide solutions are usually measured in µg/µl or ng/µl. For the calculation of the weight-to-weight ratio 1 µl of the BPP Bioportide™ solution is compared to 1 µl of the nucleic acid stock. In the standard case, the BPP Bioportide™ solution has a concentration of 100 ng/µl and the nucleic acid solution has a concentration of 10 ng/µl. The weight-to-weight ratio is calculated by dividing the concentration of the BPP Bioportide™ solution by the concentration of the nucleic acid solution. If we want to know the molar ratio, we need to include the molar weights in the calculation:

$$\left(\frac{0.1}{37500} \cdot \frac{g/l}{g/mol}\right) \Big/ \left(\frac{0.01}{Mol.\ Weight_{(ribo)nuc.}} \cdot \frac{g/l}{g/mol}\right) = molar\ ratio\ [\frac{BPP\ Bioportide^{TM}}{(ribo)nuc.\ acid}]$$

0.1 g/l equals to 100 ng/µl and 0.01 g/l equals to 10 ng/µl.