

## NPN assay

- NPN membrane permeability assay
- inoculate 20 ml of an overnight culture with 1 ml preculture
- incubate cells at 37 °C until OD<sub>600</sub> of 0.5
- centrifugation of whole culture volume (10 min at 3000 g)
- discard entire supernatant
- resuspend pellet in the same volume **PBS Buffer**
- repeat washing steps 3 to 5 twice
- mix washed cells with 10 mM NPN stock solution to a final concentration of 10 µM in a UV cuvette
- fluorescence measurement with **Shimadzu fluorescence spectrophotometer**
  - sample volume: 1 ml
  - Excitation: 355 nm
  - Emission Bandwidth: 380 - 500 nm

