β-Galactosidase Assay with CPRG

- Lyse cells
  (recommended cell lysis buffer: 0.25M Tris/HCl pH 8.0, 0.25% (v/v) NP40, 2.5 mM EDTA)

- Pipet about 10 µl of extract into a 96-well plate (appropriate neg. control / blank: 10 µl of mock transfected cells, or non-transfected cells – as there is a slight endogenous β-Gal activity) – leave one well empty for blank (A-1)

- Add 100 µl substrate solution to the wells (also to the blank-well)
- Incubate until red color develops (min to hours – depending on β-Gal activity, if you have low activity you can also incubate at 37°C)

- Optional: Stop with 50 µl of Stop solution (only necessary if you want to time it exactly, e.g. by adding the substrate in a timed way and stopping the reaction in the same way)

- Measure with ELISA Reader at 570 nm (Filter #3)

Lysis Buffer:
0.25M Tris/HCl pH 7.4 (or better 8.0)
0.25% (v/v) NP40
2.5 mM EDTA

CPRG-substrate solution: 1 mg/ml (= 1.65 mM)
in PBS + 10 mM KCl, + 1 mM $MgCl_2$
alternative substrate buffer:
60 mM Na_2HPO_4 pH 8.0, 1 mM $MgCl_2$, 10 mM KCl, 50 mM Mercapto-ethanol

Stop solution: 0.5M Na_2CO_3