New Sandwich ELISA for rapid and accurate quantification of GFP-fusion proteins in cells

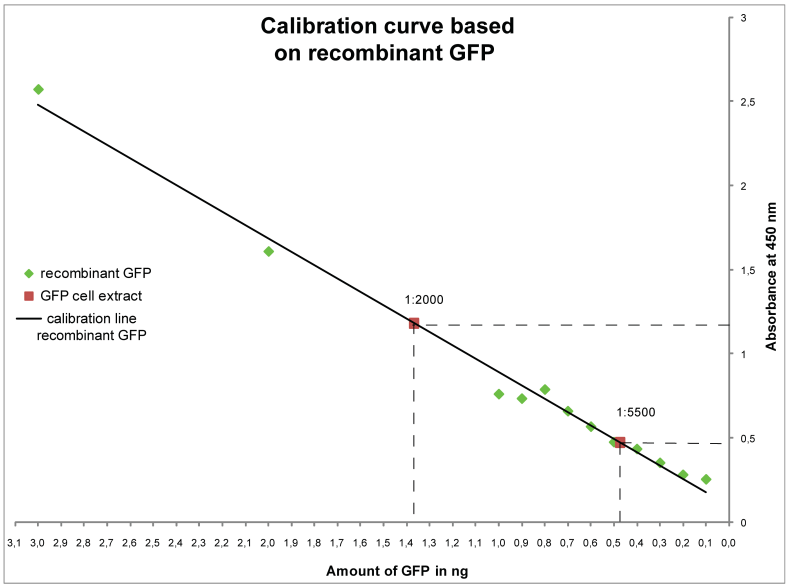
Background

The green fluorescent protein (GFP) and variants thereof are widely used to study the subcellular localization and dynamics of proteins. GFP fusion proteins can be expressed in different cell types at different expression levels by transient or stable transfection. Transient expression may provide quick informative results, however, in many cases it is necessary to generate stable cell lines that express the GFP fusion protein of interest at a level similar to the one of the endogenous protein.
Quantification of GFP fusion proteins in cells can be tricky since existing methods, like fluorescence microscopy or Western Blotting, often show insufficient signal to noise ratios or high signal discrepancies. The major challenge is to increase the sensitivity while keeping the background low. The following application note describes the accurate quantification of stably expressed GFP fusion proteins in cellular extracts using a new Sandwich ELISA comprising the GFP-multiTrap® in combination with a highly sensitive monoclonal anti-GFP antibody.

Application

The experiment was performed using purified recombinant GFP and HEK293T cells stably expressing GFP. Purified GFP was serially pre-diluted in phosphate buffered saline (PBS) to obtain a final amount of 3 ng to 0,1 ng per well. Cellular extract from 10^7 HEK293T cells was prepared as described (1). Subsequently the cell extract was serially pre-diluted from 1:500 to 1:10500 in PBS. GFP was immobilized with the GFP-multiTrap® by adding 100 µl of the diluted GFP reference or GFP cell extract to each well. For quantitative binding the GFP-multiTrap® plate was incubated for one hour at room temperature.
After six washing steps captured GFP was detected with the rat monoclonal anti-GFP-antibody (code: 3E5, 5 µg/ml, ChromoTek GmbH) in combination with a secondary anti-rat antibody coupled to horse radish peroxidase (HRP) (Jackson Immunoresearch, Cat. No. 112-035-175, 0.4 µg/ml). 3,3′,5,5′-tetramethylbenzidine (TMB) (1-Step Ultra TMB; Thermo Fisher Pierce, Cat. No. 34028) was used as chromogenic substrate for HRP. After 15 minutes the reaction was stopped by addition of 2M sulfuric acid and the OD_{450nm} was measured in a photometer.

The blank value ($OD_{450nm,}$ secondary antibody only) was subtracted and the OD_{450nm} values of the recombinant GFP reference samples were plotted against the known concentrations of these standards and a straight calibration line was obtained. Using this calibration curve one can determine the concentration of the unknown samples. Since the linear range of most analytical instruments is known to be limited, the linearity of the calibration curve was evaluated during the validation of the method and the linear range of the calibration curve was determined in three independent experiments giving reproducible results.



Cell Calculation:

Dilution of HEK293T cell extract:	0	1:500	1:1000	1:1500	1:2000	1:2500	1:3000	1:3500	1:4000	1:4500	1:5000
Number of cells per well	1 x 10^7	~ 20000	~ 10000	~ 6700	~ 5000	~ 4000	~ 3300	~ 2900	~ 2500	~ 2200	~ 2000

Dilution of HEK293T cell extract:	1:5500	1:6000	1:6500	1:7000	1:7500	1:8000	1:8500	1:9000	1:9500	1:10000	1:10500
Number of cells per well	~ 1800	~ 1700	~ 1500	~ 1400	~ 1300	~1250	~ 1200	~ 1100	~ 1050	~ 1000	~ 950

Model Calculation

According to the calibration curve, the GFP signal of the 1:5500 dilution of the HEK293T cell extract - corresponding to 1800 cells - equates to ~ 460 pg of recombinant GFP.
The total amount of GFP (460 pg) divided by the number of cells (1800) yields ~ 0,26 pg GFP per cell, which is in accordance with the data obtained from the 1:2000 dilution (corresponding to 5000 cells). The amount of GFP per cell for this example is calculated as ~ 0,27 pg GFP per cell.

Conclusion:

As can be seen from the results above, the new Sandwich ELISA comprising the versatile GFP-multiTrap® and a highly sensitive monoclonal anti-GFP antibody allows fast and reliable quantification of the absolute amount of GFP-fusion proteins per cell. The high sensitivity of the GFP-multiTrap® offers a quick and simple alternative to conventionally used methods like fluorescence microscopy or Western Blotting.

Ordering Information

Product	Quantity	Code
GFP-multiTrap®	96 well plate	gtp-96
GFP antibody [3E5]	100 µg	please inquire

http://www.chromotek.com

(1) Rothbauer, U. et al. A versatile nanotrap for biochemical and functional studies with fluorescent fusion proteins. Mol Cell Proteomics 7, 282-289 (2008).