Hit Validation and Affinity Determination Service

In some cases, peptide hits of a primary peptide microarray screening campaign - such as the search for peptide target binders or epitope mappings - need to be validated and further characterized in an independent assay format. PEPperPRINT offers the confirmation and affinity determination of your primary hit peptides to a target protein or antibody in a soluble, homogenous format using fluorescence polarization as quantitative measure for protein-peptide binding.

Fluorescence Polarization: How it Works

Assay principle: The peptide-protein or peptide-antibody affinity is determined by fluorescence polarization measurement. The degree of fluorescence polarization is highly dependent on the molecular mobility of the fluorophore, which is inversely correlated to the molecular weight of the fluorophore (Figure 1).

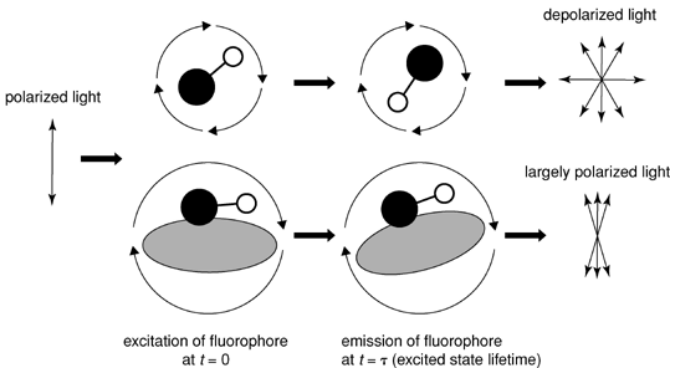

Figure 1: **Principle of fluorescence polarization.**
When a small peptide (black circle) with a fluorescent label (white circle) is excited by polarized light at the excitation wavelength of the fluorophore, the ligand reorients to a significant degree during the excited state lifetime of the fluorophore due to molecular tumbling. This causes the emitted light to be largely depolarized. If the ligand is bound to a protein (gray ellipse), the resulting complex tumbles much slower, and the emitted light retains its polarization.

Experimentally, the degree of polarization is determined by measurements of fluorescence intensities parallel and perpendicular to the plane of linearly polarized excitation light, and is expressed in terms of fluorescence polarization P (units: mP).

$$P = \frac{F_{\parallel} - F_{\perp}}{F_{\parallel} + F_{\perp}}$$

$F_{\parallel}$= fluorescence intensity parallel to the excitation plane

$F_{\perp}$= fluorescence intensity perpendicular to the excitation plane

K_d/EC_{50} Determination

The K_d/EC_{50} values are determined by monitoring the binding of the target protein or antibody to a fixed amount of the labeled peptide with increasing protein concentrations. The fluorescence polarization is measured under equilibrium conditions. A dose response curve

is generated by plotting the fluorescence polarization values against the protein concentration, and the K_d/EC_{50} values are calculated from the binding curve (Figure 2).

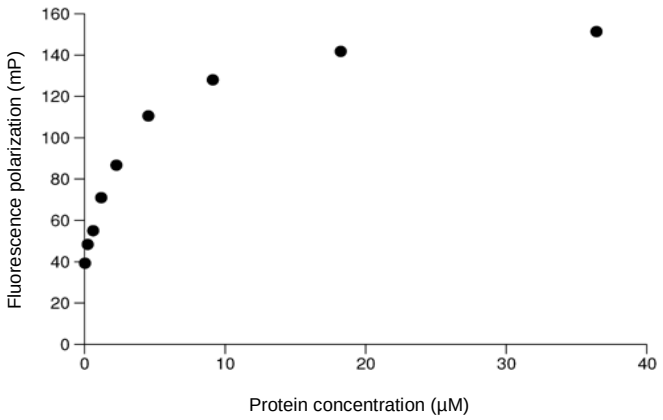

Figure 2: Dose response curve taken by fluorescence polarization.
A fixed amount of labeled peptide ligand is incubated with increasing amounts of the target protein or antibody. The resulting fluorescence polarization is measured under equilibrium conditions. In this example, the K_d of the peptide corresponds to 3 µM.

Material Requirements

Fluorescein labeled peptide: 0.5 mg of a HPLC-purified and fluorescein labeled peptide is provided by PEPperPRINT.

Target protein or antibody: To be supplied by the customer. The affinity measurement requires purified protein or antibody.

Protein or antibody need: The dose-response curve is taken at concentrations between 0.001 µM and 1µM and requires approximately 0.5 mg protein. Weaker ligands (K_d > 1 µM) are analyzed at higher protein concentrations, and for measurements of up to 10 µM 1 to 2 mg target protein are required, depending on the protein´s molecular weight. To save material, the experiments are run in low-volume 384-well plates in a final volume of 25 µl.

Report/Delivery

Report: The report comprises a description of the assay protocol, the dose-response curve and the estimated K_d or EC_{50} value of the peptide ligand against the protein or antibody target.