Cellular Phosphorylation Assay for EGF-R

REACTION BIOLOGY

CELLULAR ASSAY SERVICES

➢ The Target

Epidermal growth factor receptor (EGF-R) is a 179kDa membrane-bound glycoprotein expressed on the surface of epithelial cells. EGF-R is a member of the growth factor receptor family of protein tyrosine kinases. Ligand-dependent receptor activation results in autophosphorylation of the receptor's intracellular kinase domain. Genetic alterations which impact upon the activation level of EGF-R, result in cell proliferation. Therefore, this process is responsible for cellular transformation and makes EGF-R a preferred cancer-related therapeutic target.

➢ Cellular Phosphorylation Assay

The human epidermoid carcinoma cell line (A431) is known to overexpress EGF-R. Stimulation of these cells with its physiological ligand human epidermal growth factor (EGF), results in a robust receptor autophosphorylation. Compounds are preincubated before cell stimulation to allow thorough target binding. Stimulation conditions are optimized to determine dose-related inhibition of the phospho-EGF-R signal, which is subsequently quantified by Sandwich-ELISA technique. The assay is validated based on known inhibitors of EGF-R kinase activity (see Fig.1).

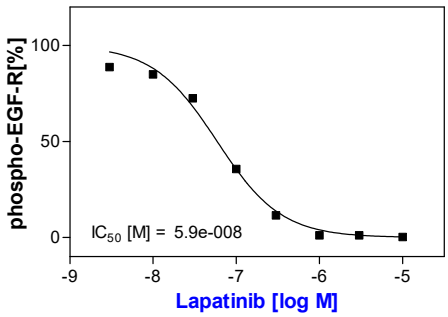
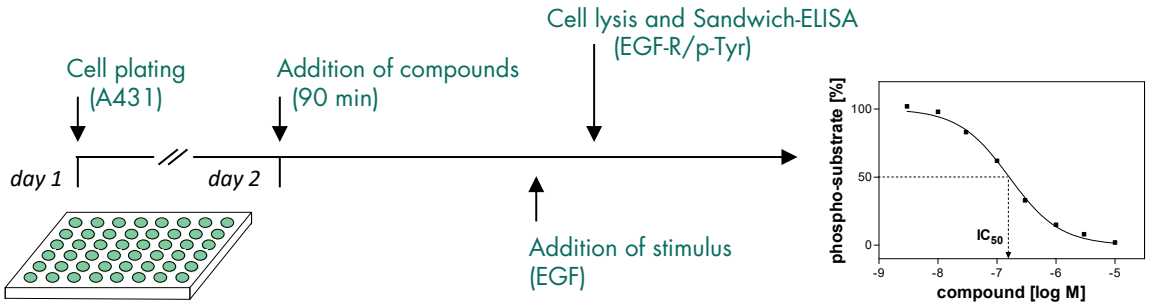


Figure 1: Assay validation.
Lapatinib is known to inhibit the EGF-induced phospho-EGF-R signal in a specific manner. The compound was included for the validation process and the cellular EGF-R assay generated highly reproducible IC_{50} values. The graph shows a representative result.

➢ You ship your compounds – Reaction Biology performs the testing



- IC_{50} values are determined by testing 8 compound concentrations in semi-logarithmic steps (each concentration in duplicates).
- Quality assurance is provided by calculation of Z' factors for Low/High controls on each assay plate and by including a full IC_{50} curve for a reference inhibitor to monitor adequate dose/response relation in your assay run.