

➤ The Target

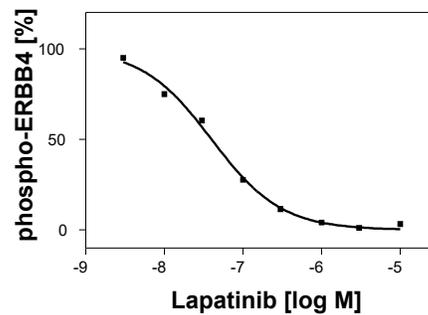
The receptor tyrosine kinase ERBB4 (Erythroblastic Leukemia Viral Oncogene Homolog-4) belongs together with EGF-R, ERBB2 and ERBB3 to the ERBB receptor family. It binds members of the neuregulin and the EGF family (NRG1-4, BTC, EREG and HBEGF). Binding of ligand results in receptor homo- or heterodimerization with EGF or ERBB2 and subsequently receptor autophosphorylation. ERBB4 signaling regulates cell proliferation, differentiation, migration and apoptosis. It plays an important role in the development of the heart, the central nervous system and the mammary gland.

➤ Cellular Phosphorylation Assay

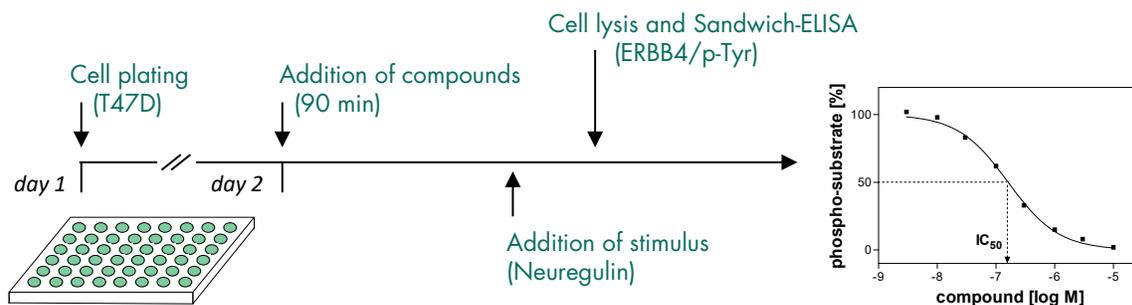
The human breast cancer cell line T47D is known to endogenously express the receptor tyrosine kinase ERBB4. Stimulation of these cells with the physiological ligand Neuregulin results in a robust receptor autophosphorylation. Compounds are preincubated before cell stimulation to allow thorough target binding. Stimulation conditions are optimized to determine dose-related inhibition of the phospho-ERBB4 signal, which is subsequently quantified by Sandwich-ELISA technique. Figure 1 shows data confirming the inhibitory activity of Lapatinib, a cognate ERBB4 inhibitor.

Figure 1: Assay validation.

Lapatinib is a potent inhibitor of the phospho-ERBB4 signal as observed in T47D breast cancer cells. The graph shows a representative result.



➤ You ship your compounds – Reaction Biology performs the testing



- IC₅₀ 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 IC₅₀ curve for a reference inhibitor to monitor adequate dose/response relation in your assay run.