A new HTRF® cell-based EGFR platform to facilitate the development of anti-cancer drugs targeting EGFR, HER2 and HER3 receptors

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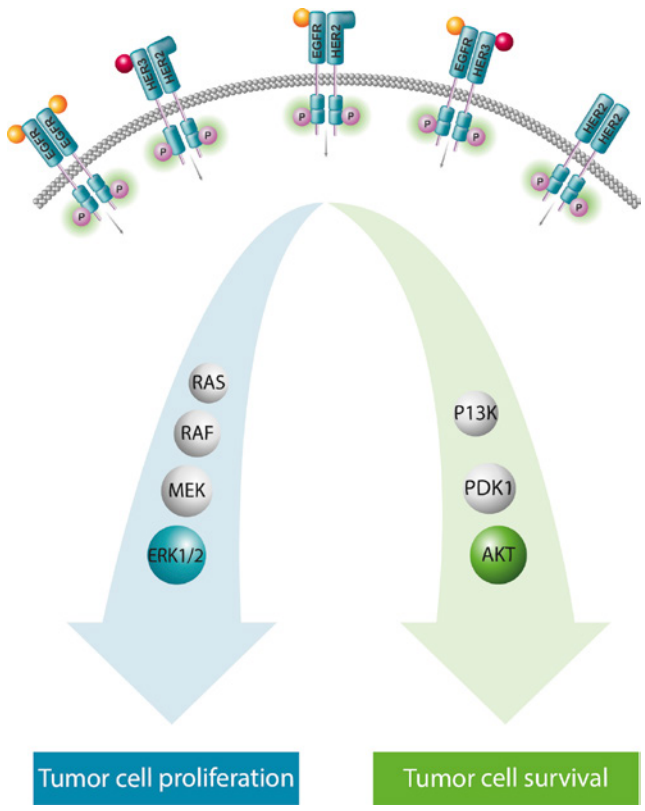
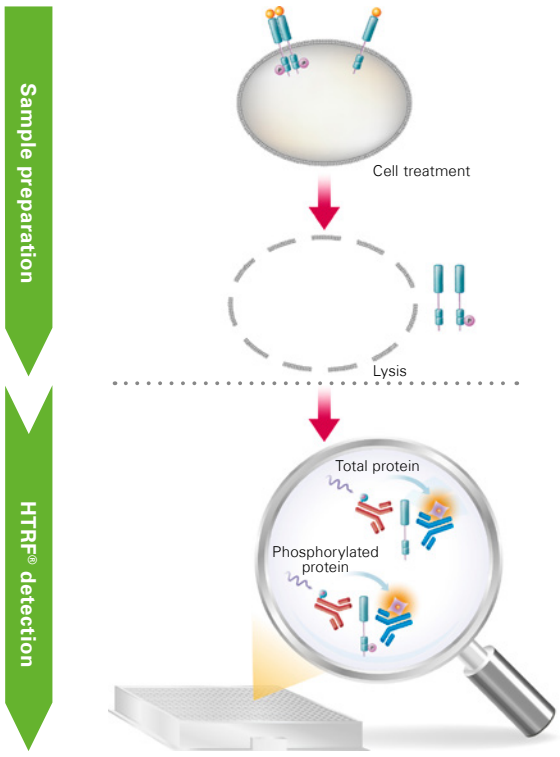
CONCEPT Receptor tyrosine kinases of the EGFR family, including HER2 and HER3, are activated by growth factor binding that induces homo- or hetero-dimerization and trans-phosphorylation of the cytoplasmic tyrosine residues. They control crucial signaling pathways (mainly MAPK/ERK and PI3K/AKT) leading to cell proliferation and survival (1). Their overexpression and/or mutation, responsible for their hyper-activation, is present in many malignant diseases such as breast, ovarian, lung and colorectal carcinomas. One of the main strategies to target these receptors for cancer treatment is the use of Tyrosine Kinase Inhibitors (TKIs) that inactivate the intracellular kinase domain. A second approach is based on the development of monoclonal Antibodies (mAbs) that interfere with the extracellular domain, thereby preventing activation and dimerization transmitted by growth factors (2).

To facilitate the discovery of such anti-cancer drugs while avoiding the use of conventional time-consuming techniques like Western Blot and ELISA, Cisbio Bioassays has developed 6 new HTRF® cell-based assay kits to monitor the phosphorylation of EGFR (Y1068), HER2 (Y1221/1222) and HER3 (Y1289), as well as their expression levels. With a fast and simple no-wash protocol, these homogeneous sandwich assays based on TR-FRET enable the rapid and direct detection of endogenous receptors in cells or tissue. Here we present the validation of these novel assays on different human tumor cell lines, using either TKIs or mAbs.



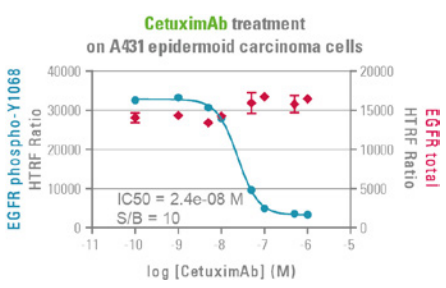
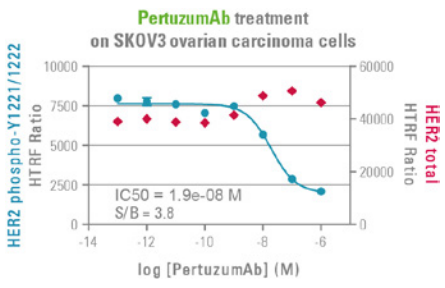
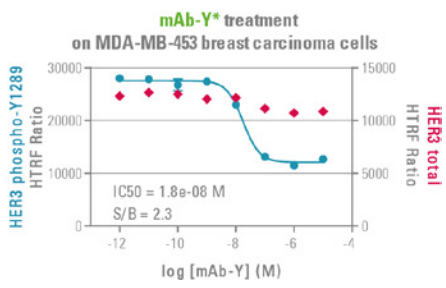
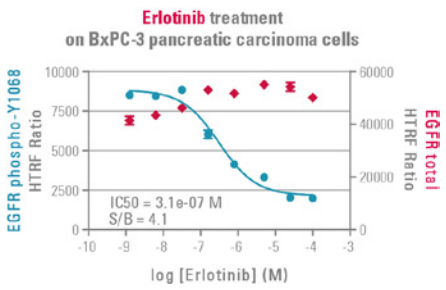
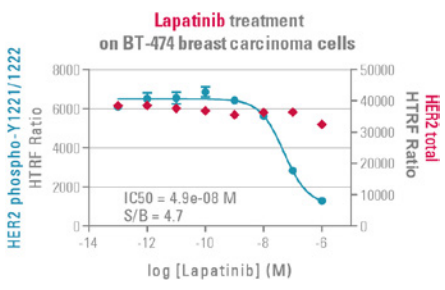
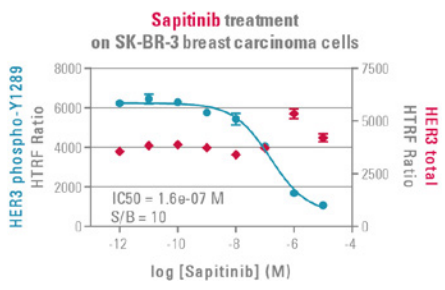
ASSAY PRINCIPLE



Kit reagents (Premixed donor & acceptor antibodies)

ASSAYS IN ACTION USING BIOTHERAPEUTICS AND SMALL MOLECULES

Dose-response experiments with pharmacological inhibitors were performed on different human tumor cell lines. After cell lysis, the same lysate was transferred to the detection plate twice to analyze in parallel both the phosphorylation status of the receptor and its corresponding total protein level. The HTRF® signal was recorded on a PHERAstar FS reader (flash lamp).

	EGFR-TARGETED DRUG	HER2-TARGETED DRUG	HER3-TARGETED DRUG
+ mAbs			
	50,000 cells + CetuximAb (30 min) + 80 nM EGF (10 min)	100,000 cells + PertuzumAb (30 min) + 100 nM EGF (10 min)	100,000 cells + mAb-Y (30 min) + 100 nM Heregulin-β1 (10 min) * mAb-y was kindly provided by T. Chardès
+ TKIs			
	50,000 cells + Erlotinib (4 hours) + 150 nM EGF (5 min)	100,000 cells + Lapatinib (3 hours)	100,000 cells + Sapitinib (30 min)

CONCLUSION Cisbio Bioassays has developed new HTRF® cell-based phospho-/total assays to support the screening and the characterization of anti-cancer agents directed against EGFR, HER2 and HER3.

Their pharmacological validation using reference TKIs and mAbs shows that all of the 6 assays have the sensitivity and the accuracy required to detect endogenous receptors in commonly used cancer cell lines and to determine pharmacological parameters as IC_{50} values.

Thanks to their very easy and rapid « add and read » protocol, these tools represent a real improvement over more conventional heterogeneous methods for the development of anti-tumor drugs. Moreover, they can be easily combined with the already existing HTRF® cellular phospho-/total ERK and AKT assays to study the effect of potential anti-tumor drugs on downstream signaling pathways.

REFERENCES

1. Zaczek et al., Histol Histopathol (2005) 20: 1005-1015
2. Scaltriti and Baselga, Clin Cancer Res (2006) 12: 5268–5272

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