Technical Data Sheet

Research Use Only. Not for use in diagnostic procedures.

Neo Biotech Phospho-ERK1/2 (T202/Y204) + Total-ERK1/2 TR-FRET Cellular Assay Kit

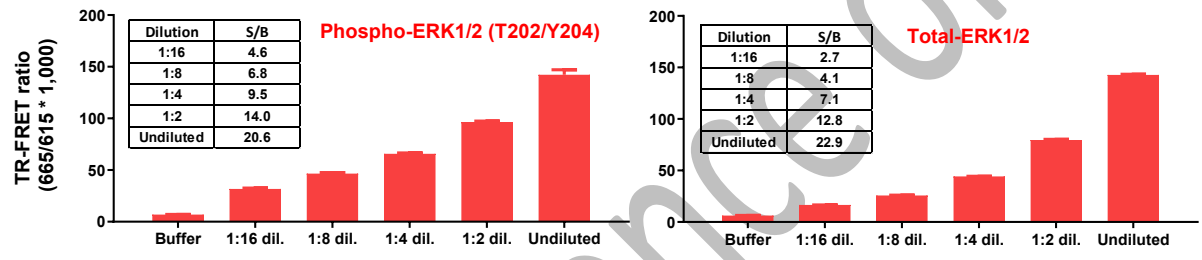
For products NB-41-JNKT-100

Kit-specificity

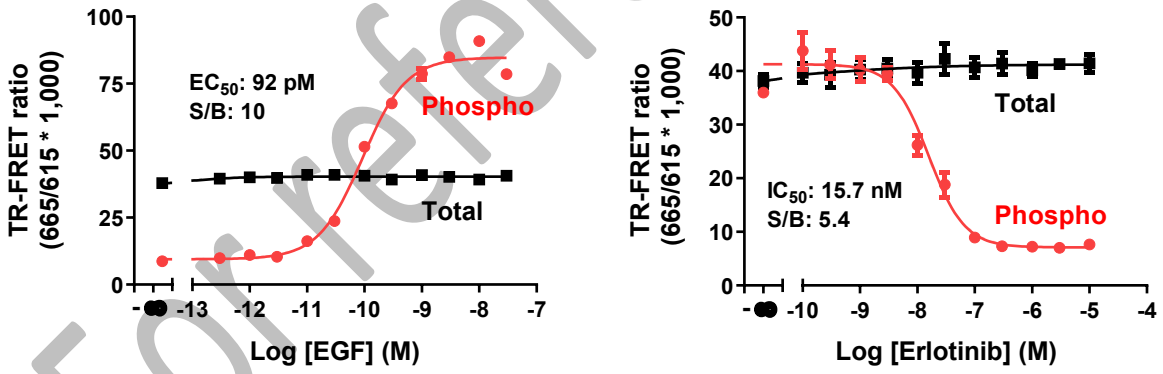
The proteins detected by this kit correspond to Swiss-Prot Acc. P27361 and P28482, and Entrez-Gene Id 5595 and 5594. This assay kit contains 2 highly specific and selective antibody pairs, one that recognizes phospho-ERK1/2 (Thr202/Ty204) and another that recognizes ERK1/2. These antibodies recognize ERK1/2 of human, mouse, rat, mink, monkey, bovine, pig and zebrafish origin. Other species should be tested on a case-by-case basis.

Control lysate

HEK293 cells were grown in a T175 flask at 37°C, 5%CO_2, in EMEM with 10% FBS and cultured to 90% confluence. The medium was removed and cells were stimulated with 30 nM of EGF for 10 min. The medium was removed and cells were lysed with 4 mL of 1X Lysis Buffer #1 containing 1 mM NaF and 2 mM Na_3VO_4, on an orbital shaker (400 rpm) for 15 min at room temperature. Soluble supernatants were collected after a 10 min centrifugation and tested using the two-plate assay protocol.



Typical data generated with the two-plate assay protocol



HEK293 cells were seeded at 50,000 cells/well and cultured overnight at 37°C, 5% CO_2, in EMEM with 10% FBS. Cells were then treated with either EGF for 15 min (stimulation) or with erlotinib for 15 min then EGF (inhibition). The media was removed from the wells, and cells were lysed with 50 µL/well of 1X Lysis Buffer #1 containing 1 mM NaF and 2 mM Na_3VO_4, on an orbital shaker (400 rpm) for 15 min at room temperature. The lysates were then analyzed on separate wells for phospho-ERK1/2 (T202/Y204) and total-ERK1/2 using the two-plate assay protocol. The TR-FRET signal was recorded at 665 and 615 nm (EnVision®; lamp excitation) after a 4 h incubation period at room temperature.

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