



User's Manual

Phospho-FGFR2 (Tyr653/654) Kit



DEIA156V2



500T



This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

This assay is intended for the simple, rapid and direct detection of endogenous levels of FGFR2 in cells, only when phosphorylated at Tyr653/654. Upon activation, FGFR2 is phosphorylated and after lysis of the cell membrane, Phospho-FGFR2 (SerY653/654) can be detected using the kit reagents.

Principles of Testing

As shown here, Phospho-FGFR2 (SerY653/654) is detected in a sandwich assay format using 2 different specific antibodies, one labelled with Eu3+-Cryptate (donor) and the second with d2 (acceptor).

When the dyes are in close proximity, the excitation of the donor with a light source (laser or flash lamp) triggers a Fluorescence Resonance Energy Transfer (FRET) towards the acceptor, which in turn fluoresces at a specific wavelength (665 nm). The specific signal modulates positively in proportion to Phospho- FGFR2 (SerY653/654).

The assay can be run under a two-plate assay protocol, where cells are plated, stimulated and lysed in the same culture plate. Lysates are then transferred to the assay plate for the detection of Phospho-FGFR2 (SerY653/654) by HTRF® reagents. This protocol gives the cells viability and confluence to be monitored. It can also be further streamlined to a one-plate assay protocol. Detection of Phospho-FGFR2 (SerY653/654) with HTRF® reagents is performed in a single plate used for plating, stimulation and detection. No washing steps are required. This protocol, HTS designed, allows miniaturization while maintaining HTRF® quality.

Reagents And Materials Provided

1. Phospho-FGFR2 Cryptate antibody
2. Phospho-FGFR2 d2 antibody
3. Control lysate (ready-to-use)
4. Blocking reagent* (stock solution 100X)
5. Lysis buffer* # 2 (stock solution 4X)
6. Detection buffer (ready-to-use)

Storage

Antibodies, control lysate and buffers should be stored frozen until use.

Thawed lysis buffer, detection buffer and blocking reagent can be stored at 2-8°C in your premises.

Thawed antibodies are stable 48 hours at 2-8°C; they can be refrozen (at -20°C or below) and thawed at least one more time.

Control lysate must be stored frozen at -60°C or below. Thawed control lysate can be refrozen (at -60°C or below) and thawed one more time.