TECHNICAL DATA SHEET

THUNDER™ Phospho-SYK (Y525/Y526) + Total SYK TR-FRET Cell Signaling Assay Kit

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CATALOG NUMBERS KIT-SYKPT-500 (500 tests)
400 points for phospho-SYK
and 100 points for total SYK

Store at -80°C
For research use only.
Not for use in diagnostic procedures.

PRODUCT DESCRIPTION

This assay kit measures intracellular levels of **phospho-SYK (Y525/Y526) and total SYK** protein in cell lysates using a simple, rapid and sensitive immunoassay based on the homogeneous (no-wash) THUNDER™ TR-FRET technology. The kit is compatible with both adherent and suspension cells.

SPECIFICITY

This assay kit contains two specific and selective antibody pairs, one that recognizes **SYK** phosphorylated at **Tyr525/Tyr526**, and another that recognizes **total** (both phosphorylated and unphosphorylated) **SYK**.

SPECIES REACTIVITY

Human (Swiss-Prot Acc.: P43405; Entrez-Gene Id: 6850).

Other species should be tested on a case-by-case basis.

TR-FRET ASSAY PRINCIPLE

The **Phospho-SYK (Y525/Y526) + total SYK** assay kit is a homogeneous time-resolved Förster resonance energy transfer (TR-FRET) sandwich immunoassay (Figure 1). The THUNDER™ Cell Signaling assay workflow consists of 3 steps (Figure 2). Following cell treatment, cells are first lysed with the specific Lysis Buffer provided in the kit. Then **Phospho-SYK (Y525/Y526) and total SYK** are detected in the cell lysates in separate wells with two pairs of fluorophore-labeled antibodies in a simple "add-incubate-measure" format (single-step reagent addition; no wash steps). For detection of the phosphorylated protein, one antibody is labeled with a donor fluorophore (Europium chelate; Eu-Ab1) and the second with a far-red acceptor fluorophore (FR-Ab2). The same approach is used for the second antibody pair detecting the total protein (Eu-Ab3 and FR-Ab4). The binding of the two matched labeled antibodies to distinct epitopes on the target protein (either **phospho-SYK** or **total SYK**) takes place in solution and brings the two dyes into close proximity. Excitation of the donor Europium chelate molecules with a flash lamp (320 or 340 nm) or a laser (337 nm) triggers a FRET from the donor to the acceptor molecules, which in turn emit a TR-FRET signal at 665 nm. Residual energy from the Eu chelate generates light at 615 nm. The signal at 665 nm is proportional to the concentration of **Phospho-SYK (Y525/Y526)** and **Total SYK** in the cell lysate. Data can be expressed as either the signal at 665 nm or the 665 nm/615 nm ratio.

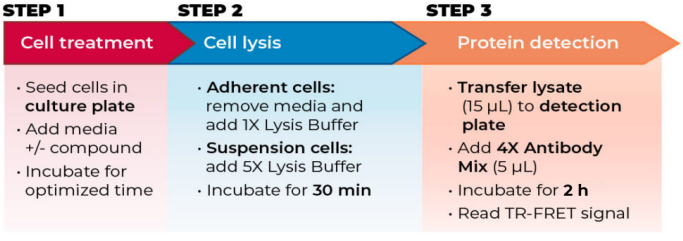


Figure 2 Assay workflow using the 2-plate (transfer) protocol.

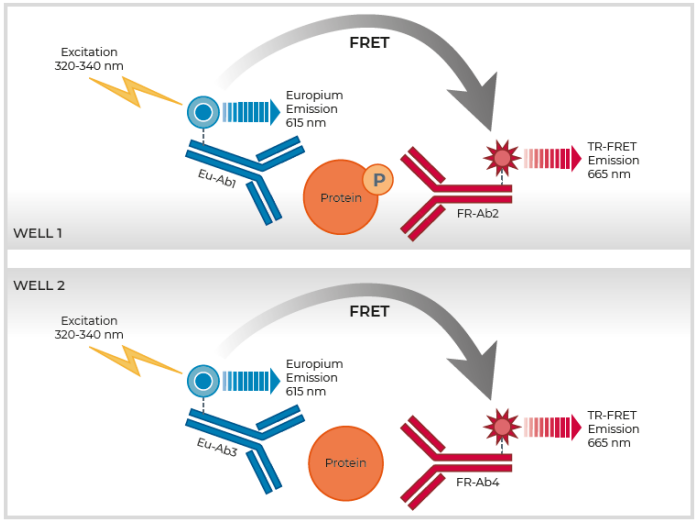


Figure 1 Schematic representation of the TR-FRET cell signaling assay principle.

KIT COMPONENTS

	500 points*
Eu-labeled phospho-SYK (Y525/Y526) antibody (Eu-Ab1)	20 µL
Acceptor-labeled phospho-SYK (Y525/Y526) antibody (FR-Ab2)	80 µL
Eu-labeled total-SYK antibody (Eu-Ab3)	5 µL
Acceptor-labeled total-SYK antibody (FR-Ab4)	20 µL
Lysis Buffer 3 (5X)	5 mL
Detection Buffer (10X)	250 µL
Positive control cell lysate	200 µL
Phosphatase Inhibitor Cocktail (100X)	250 µL

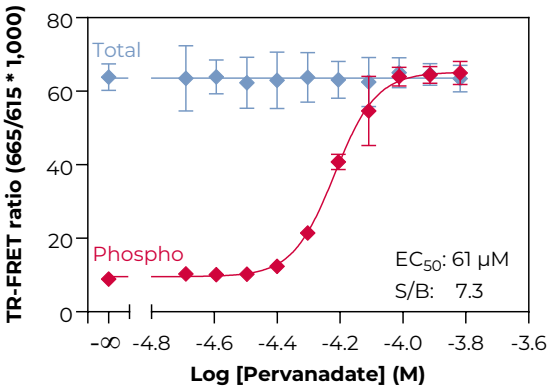
* The number of assay points is based on an assay volume of 20 µL in half-area 96-well or low-volume 384-well assay plates using the kit components at the recommended concentrations (refer to the User Manual).

VALIDATION DATA

This assay kit has been validated for the relative quantification of phospho-SYK (Y525/Y526) and total SYK in Raji and U-937 cell lysates using the 2-plate assay protocol.

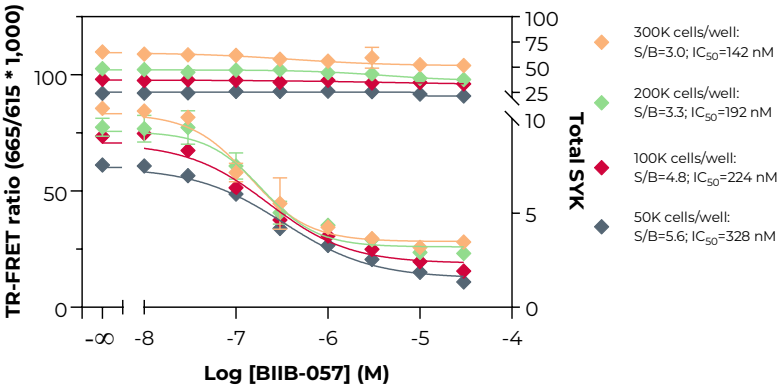
- Non-adherent cells were cultured in RPMI+10% FBS before being centrifuged and resuspended at the desired density in RPMI without serum.
- Following cell treatment, cells were lysed with the 5X **Lysis Buffer 3** supplemented with the 100X Phosphatase Inhibitor Cocktail diluted at 5X.
- Following a **30-min** incubation at room temperature (RT) on an orbital shaker (400 rpm), lysates (15 µL) were then transferred to a 384-well assay plate followed by addition to separate wells of either the labeled antibodies Eu-Ab1 and FR-Ab2 (5 µL) for detection of phospho-SYK (Y525/Y526) or Eu-Ab3 and FR-Ab4 (5 µL) for detection of total SYK.
- The plate was incubated at RT for **2 hours** and the TR-FRET signal was recorded at 665 and 615 nm (EnVision®; laser excitation).

STIMULATION OF PHOSPHO-SYK (Y525/Y526) IN RAJI CELLS



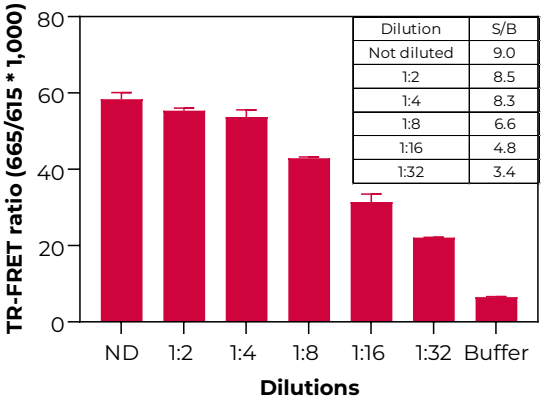
Raji cells (100,000 cells/well; in triplicate) were incubated with serial dilutions of pervanadate for 30 min at 37°C. Data show that treatment of Raji cells with pervanadate stimulates phosphorylation of SYK at Y525/Y526 but does not affect the levels of Total SYK.

INHIBITION OF PHOSPHO-SYK (Y525/Y526) IN U-937 CELLS



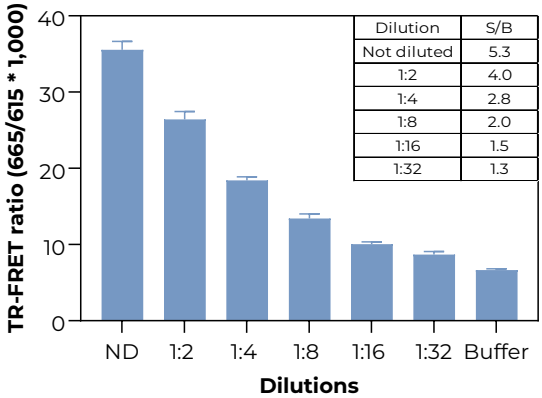
U-937 cells (50,000 to 300,000 cells/well; in triplicate) were incubated with serial dilutions of BIIB-057 for 60 min at 37°C. Cells were then stimulated with 200 µM of pervanadate for 30 min at 37°C. Data show that treatment of U-937 cells with BIIB-057 inhibits phosphorylation of SYK at Y525/Y526 by pervanadate but does not affect the levels of Total SYK.

U-937 CONTROL LYSATE TITRATION (QC TEST) PHOSPHO-SYK (Y525/Y526)



Dilution	S/B
Not diluted	9.0
1:2	8.5
1:4	8.3
1:8	6.6
1:16	4.8
1:32	3.4

U-937 CONTROL LYSATE TITRATION (QC TEST) TOTAL SYK



Dilution	S/B
Not diluted	5.3
1:2	4.0
1:4	2.8
1:8	2.0
1:16	1.5
1:32	1.3

Quality Control: the Phospho-SYK (Y525/Y526) + Total SYK assay kit is routinely tested against pervanadate-treated U-937 lysates. U-937 cells were cultured in a T175 flask, centrifuged and resuspended at 10 million cells/mL, and stimulated with 200 µM of pervanadate for 30 min at 37°C. Following cell lysis using 1X Lysis Buffer 3, lysates were serially diluted with 1X Lysis Buffer 3 and tested in triplicate and in separate wells for phospho-SYK (Y525/Y526) and total SYK. Data show a linear relationship between lysate dilutions and TR-FRET ratio values. Note that due to the very high sensitivity of the Phospho-SYK kit, lysates from the T175 flask required at least a 1:4 pre-dilution in order to be within the dynamic assay range.

FOR MORE INFORMATION ON DEVELOPING AND OPTIMIZING TR-FRET CELL SIGNALING ASSAYS, CONSULT THE USER MANUAL.

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