

Time-resolved Förster Resonance Energy Transfer Assays for Measurement of Endogenous Phosphorylated STAT Proteins in Human Cells

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Citation

 Padros, J., Chatel, G., Caron, M. Time-resolved Förster Resonance Energy Transfer Assays for Measurement of Endogenous Phosphorylated STAT Proteins in Human Cells. *J. Vis. Exp.* (175), e62915, doi:10.3791/62915 (2021).

Date Published

September 9, 2021

DOI

10.3791/62915

URL

jove.com/video/62915

Materials

| Name | Company | Catalog Number | Comments |
|--|------------------------|----------------|---|
| 96-well microplate, clear, flat bottom, polystyrene, tissue culture-treated, sterile | Corning | 3595 | This is the plate for culturing cells when using the two-plate assay protocol. Other cell culture 96-well plates can be used |
| 384-well microplate, white, low-volume | PerkinElmer | 6007290 | This is the plate for TR-FRET detection when using the two- or one-plate assay protocols. Other low-volume, white 384-well plates can be used |
| A431 cell line | ATCC | CRL-1555 | |
| Adhesive microplate seal | PerkinElmer | 6050185 | |
| DMSO | Fisher | D159-4 | |
| Dulbecco's modified Eagle medium (DMEM) | Wisent | 319-005-CL | THUNDER TR-FRET is compatible with culture medium containing phenol red |
| EnVision Xcite Multilabel plate reader | PerkinElmer | 2104-0020A | The assays can be performed on a variety of plate readers equipped with the TR-FRET option |
| Erlotinib hydrochloride | Sigma | CDS022564 | |
| Falcon tissue culture treated flasks | Fisher | 13-680-65 | |
| Fetal bovine serum (FBS) | Wisent | 098-150 | |
| HeLa cell line | ATCC | CCL-2 | |
| JAK Inhibitor 1 | Cayman Chemical | 15146 | |
| Orbital plate shaker | Many options available | Not applicable | |
| Recombinant human EGF | PeproTech | AF-100-15 | |
| Recombinant human IFN α 2b | ProSpec | CYT-460 | |
| Recombinant human IL-4 | R&D Systems | 204-IL | |
| Roswell Park Memorial Institute 1640 medium (RPMI) | Wisent | 350-007-CL | THUNDER TR-FRET is compatible with culture medium containing phenol red |

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|---|----------------------|-----------------|--|
| THUNDER Phospho-STAT1 (Y701) + Total STAT1 TR-FRET Cell Signaling Assay Kit | BioAuxilium Research | KIT-STAT1PT-500 | |
| THUNDER Phospho-STAT3 (Y705) + Total STAT3 Cell Signaling Assay Kit | BioAuxilium Research | KIT-STAT3PT-500 | |
| THUNDER Phospho-STAT4 (Y693) TR-FRET Cell Signaling Assay Kit | BioAuxilium Research | KIT-STAT4P-500 | |
| THUNDER Phospho-STAT5 (Y694/ Y699) + Total STAT5 TR-FRET Cell Signaling Assay Kit | BioAuxilium Research | KIT-STAT5PT-500 | |
| THUNDER Phospho-STAT6 (Y641) + Total STAT6 TR-FRET Cell Signaling Assay Kit | BioAuxilium Research | KIT-STAT6PT-500 | |
| Trypsin/EDTA 0.05% | Wisent | 325-542-CL | |
| U266B1 cell line | ATCC | TIB-196 | |
| Ultrapure water | NA | NA | Use Milli-Q grade water (18 MΩ.cm) to dilute Lysis Buffer and Detection Buffer |