Novel and highly sensitive readouts for Toll-like Receptor (TLR) activation in endogenous cells: Specific detection of IRF5 Phospho (S446) and Dimer with AlphaLISA™ *SureFire® Ultra™* technology

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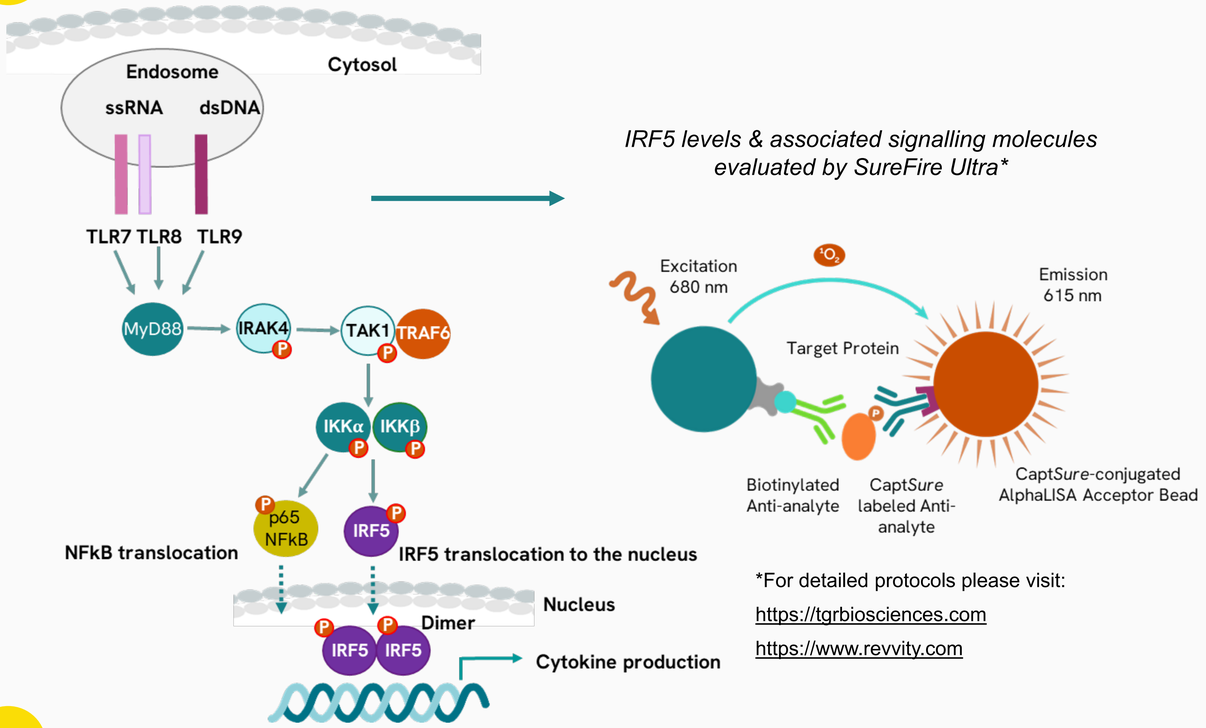
1 Overview

Interferon regulatory factor 5 (IRF5) is a key transcription factor for the activation of the pathogen-induced innate and acquired immune responses. IRF5 exists in an inactive monomeric form in the cytosol. Following TLR activation IRF5 undergoes conformational changes prior to phosphorylation and dimerization after which it is translocated to the nucleus regulating the transcription of pro-inflammatory cytokines. Deregulation of IRF5 can lead to the unrestricted production of interferons leading to numerous inflammatory and autoimmune diseases. As a result, IRF5 has emerged as a key therapeutic target for arresting the pathogenesis associated with an unregulated IRF5 pathway.

The AlphaLISA™ *SureFire® Ultra™* platform is well known for measuring protein phosphorylation and downstream cascade activation in endogenous cell systems. Here we showcase the first in-class assays for the intracellular detection of Phospho (S446) & Total IRF5 as well as an assay specifically measuring IRF5 dimer/aggregate. The induction of endogenous IRF5 phosphorylation and dimerization was measured in RPMI 8226 cells in response to R848 mediated activation of TLR7/8 and cytosine guanosine dinucleotide (CpG) mediated activation of TLR9. The phosphorylation of many other associated signalling molecules was also assessed as part of this work including NFκB, IKKα, IKKβ and TAK1.

2 Signalling Pathway



*IRF5 levels & associated signalling molecules evaluated by SureFire Ultra**

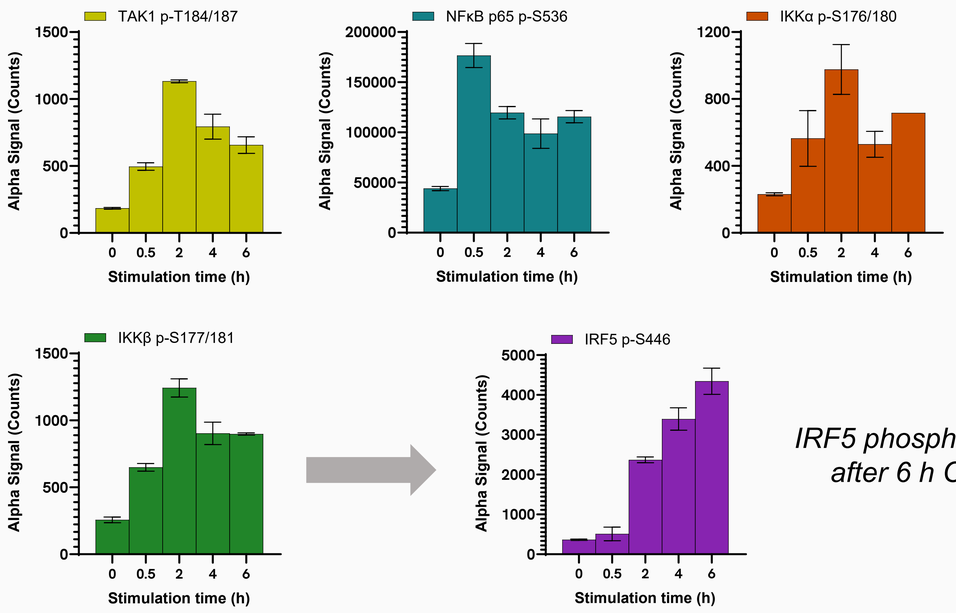
*For detailed protocols please visit:

https://tgrbiosciences.com

https://www.revvity.com

3 Results

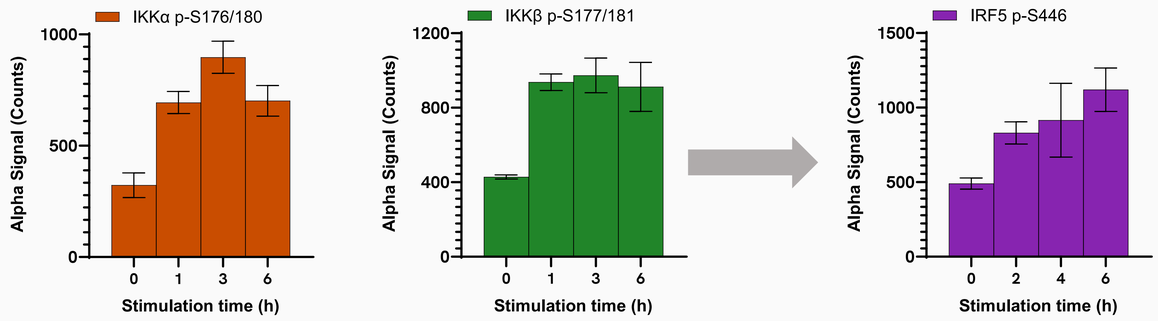
CpG mediated activation of TLR9 induces IRF5 phosphorylation in RPMI 8226 cells



IRF5 phosphorylation peaked after 6 h CpG treatment

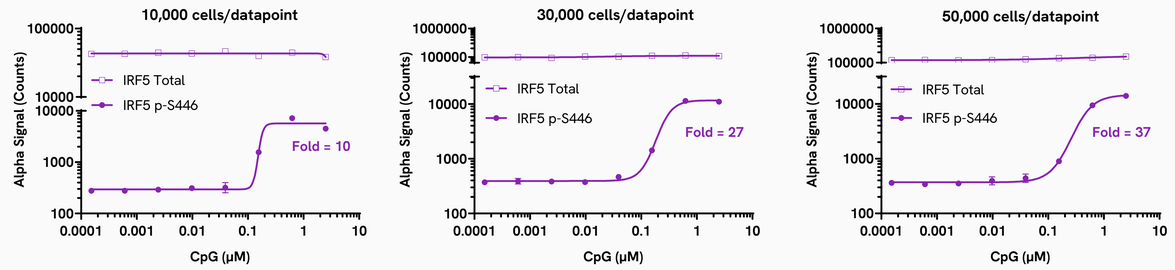
- RPMI 8226 cells treated with 1 μM CpG (ODN-2006) for various time points. Cells washed and lysed.
- A single lysate used to measure a wide range of targets using respective *SureFire Ultra* assays.
- Approximately 20,000 cells/datapoint.

R848 mediated activation of TLR7/8 induces IRF5 phosphorylation in RPMI 8226 cells



- RPMI 8226 cells treated with 1 μM R848 for various time points. Cells washed and lysed.
- A single lysate was used to measure multiple intracellular proteins by *SureFire* (20,000 cells/datapoint).

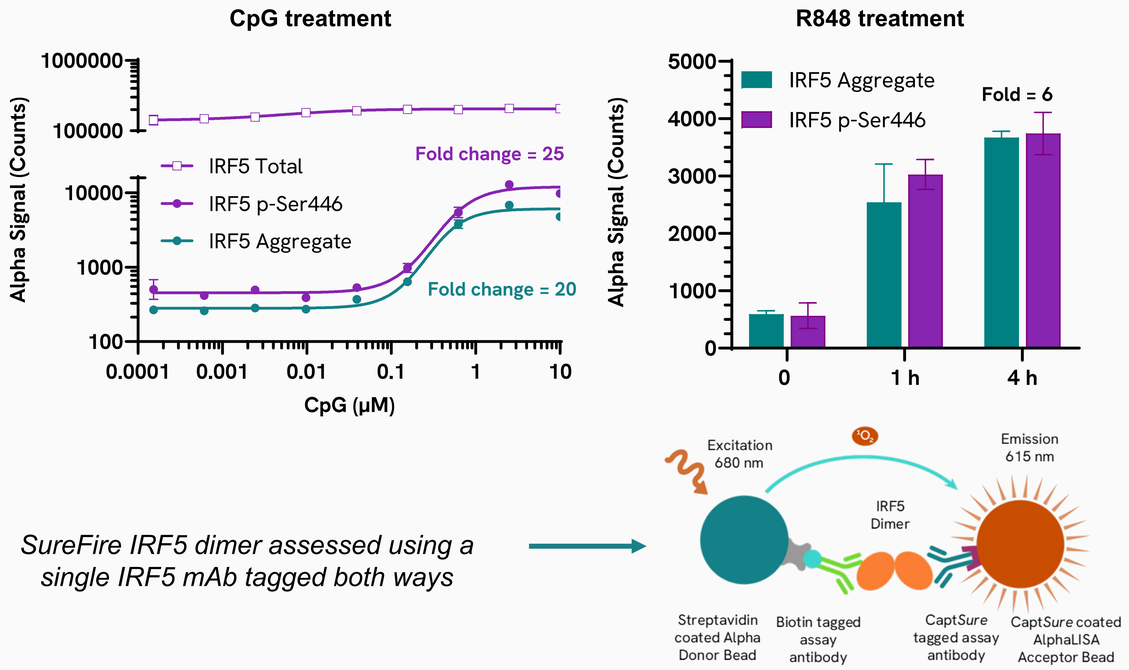
Optimising cell density to maximise the IRF5 (S446) phosphorylation window



Best signal window using 50,000 cells/datapoint

- RPMI 8226 cells treated with CpG (ODN-2006) at various concentrations for 6 h.
- Cells washed and lysed - single lysate used to evaluate Phospho/Total IRF5.

TLR activation induces IRF5 phosphorylation followed by dimerization



SureFire IRF5 dimer assessed using a single IRF5 mAb tagged both ways

4 Conclusions

- Here-in we describe the development of novel and highly sensitive Alpha *SureFire* assays that specifically measure Phospho (S446) & Total IRF5 as well as IRF5 dimer/aggregate in an endogenous cell system.
- These collective data highlight the utility of Alpha *SureFire* technology with IRF5 signalling biology and its broader value as a cell-based assay platform adaptable to basic research, drug discovery, high throughput screening, pre-clinical and clinical development.