

Homogeneous, chemiluminescent kinase assay kits for assessing the functional activity of a broad range of serine-threonine and tyrosine kinases

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Competitive binding assays have been developed for measuring the functional activity of a range of serine-threonine and tyrosine kinases. Based on enzyme fragment complementation and the subsequent hydrolysis of chemiluminescent substrate, HitHunter™ Kinase Assay Kits generate a signal that can be read either on high-throughput imaging platforms or on standard microplate readers.

Introduction

Human protein kinases are involved in numerous and diverse cellular processes such as signal transduction and cell cycle control. The precise control of protein phosphorylation is fundamental to normal cellular behaviour and uncontrolled signaling by kinases has been implicated in disease states such as cancer, atherosclerosis, psoriasis, and in inflammatory responses such as septic shock. Inhibitors that block the activity of protein kinases and phosphatases are therefore potentially useful for the development of therapeutic agents.

HitHunter Kinase Assay Kits are competitive binding assays utilizing enzyme fragment complementation (EFC) for the sensitive and simple measurement of a range of biologically important targets. Based on proprietary kinase antibody/substrate pairs, they provide a sensitive and homogeneous method for measuring the activity of a broad range of serine-threonine and tyrosine kinases covering 80% of the kinome.

HitHunter Kinase Assay principle

EFC technology is based on the recombination of two inactive β -galactosidase (β -gal) fragments; complementation of the enzyme donor (ED) fragment and the enzyme acceptor (EA) fragment forms the active tetrameric β -gal (Fig 1). Subsequent hydrolysis of luminescent substrate generates a signal that can be

easily read on standard microplate readers and imaging platforms such as the LEADseeker™ Multimodality Imaging System.

HitHunter Kinase Assay Kits utilize an ED-phosphorylated substrate conjugate that is capable of reassociation with an EA fragment to form active enzyme, but is also recognized by phosphorylated substrate antibodies. In the assay, phosphorylated substrate antibody is optimally titrated to bind ED-phosphorylated substrate conjugate and inhibit enzyme formation. However, in the presence of phosphorylated substrate from a kinase reaction, this substrate will be bound by the antibody and unbound ED-phosphorylated substrate conjugate is free to complement EA and form active enzyme. Subsequent hydrolysis of the chemiluminescent substrate by β -gal results in a positive signal directly proportional to the concentration of phosphopeptide generated by the kinase enzyme.

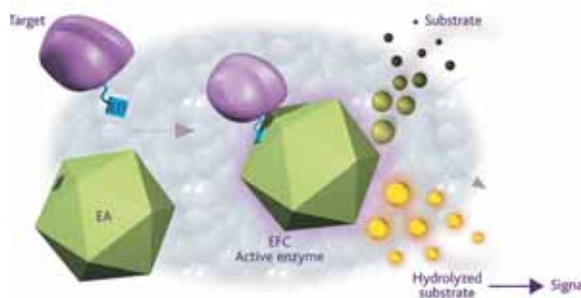


Fig 1. Principle of EFC technology.

Each HitHunter EFC Kinase Assay Kit has been developed with proprietary kinase antibodies and corresponding substrates to assess functional activity of a broad array of serine-threonine and tyrosine kinases capable of phosphorylating different kinase substrates (Table 1).

Table 1. Kinase targets and associated substrate sequences.

| Serine/threonine kinase | Sequence of peptide substrate | Kinase family |
|--------------------------------|-------------------------------|---------------|
| Crosstide | GRPRTS-pS-FAEG | AGC Grp III |
| PKC19-31 | RFARKG-pS-LRQKNV | AGC Grp III |
| Kemptide | LRRA-pS-LG | AGC Grp IV |
| Syntide 2 | PLARTL-pS-VAGLPGKK | CaMK Grp I |
| Glycogen synthase (GS) peptide | KKKNRTL-pS-VA | CaMK Grp II |
| AMARA | AMARAA-pS-VA | CaMK Grp III |
| ATF2 | VADQTP-pT-PTRFLK | CMGC |
| CHKtide | KKKVSRSGLYRSP-pS-MPENLNRRP | CMGC Grp IV |
| Myelin basic protein | FFKNIVTPR-pT-PPPSQ GK | CMGC Grp III |
| Tyrosine kinase | Sequence of peptide substrate | |
| PTK for tyrosine kinases | EE-pY-EE | TK |
| P34CDC2 | KVEKIGEGT-pY-GWY | TK |

Assay design

HitHunter Kinase Assay Kits incorporate two simple reagent additions for EFC detection following the kinase reaction (Fig 2).

Kinase reactions comprise a kinase substrate, ATP, antibody, compound, and any cofactor required to activate phosphorylation. Because reaction components are specific to each kinase, the components and incubation time for the kinase should be optimized.



Fig 2. HitHunter EFC Kinase Assay Kits protocol.

Performance

HitHunter Kinase Assay Kits produce standard curves that are 7- to 10-fold above background, resulting in highly robust assays (Z' factors of > 0.7). A typical standard curve exhibits a concentration-dependent increase in the chemiluminescent signal (Fig 3).

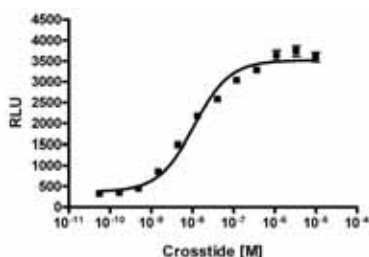


Fig 3. Typical standard curve generated on LEADseeker Multimodality Imaging System for phosphorylated crosstide substrate peptide.

For the AGC Group III kinase Akt1/PKB α , crosstide substrate was used at 25 μ M, ATP at 100 μ M, and CaCl $_2$ was added to the kinase reaction mix at 2 mM (Fig 4).

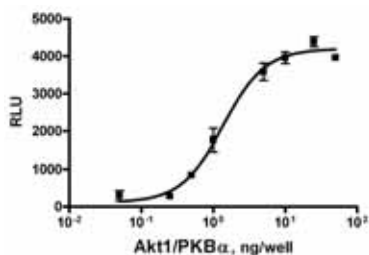


Fig 4. Akt1/PKB α kinase activity, generated on LEADseeker Multimodality Imaging System with Crosstide HitHunter Kinase Assay Kit.

Inhibition of the activity of the calcium/calmodulin-dependent kinase, CaM kinase II with the generic kinase inhibitor staurosporine was also demonstrated (Fig 5).

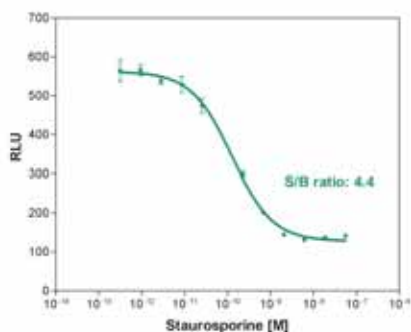


Fig 5. Staurosporine inhibition of CaM kinase II activity.

Summary

HitHunter Kinase Assay Kits enable the sensitive and specific measurement of functional activity in a broad range of serine-threonine and tyrosine kinases. The nonradioactive and homogeneous format enables miniaturization and automation and the chemiluminescent signal reduces compound interference.

Ordering information

| HitHunter Kinase Assay Kit - GS peptide | Code number |
|---|-------------|
| 100 (96 well) | 90-0059-01 |
| 800 (384 well) | 90-0059-02 |
| 10 000 (384 well) | 90-0059-03 |
| 40 000 (384 well) | 90-0059-04 |

| HitHunter Kinase Assay Kit - AMARA | Code number |
|------------------------------------|-------------|
| 100 (96 well) | 90-0060-01 |
| 800 (384 well) | 90-0060-02 |
| 10 000 (384 well) | 90-0060-03 |
| 40 000 (384 well) | 90-0060-04 |

| HitHunter Kinase Assay Kit - Syntide 2 | Code number |
|--|-------------|
| 100 (96 well) | 90-0061-01 |
| 800 (384 well) | 90-0061-02 |
| 10 000 (384 well) | 90-0061-03 |
| 40 000 (384 well) | 90-0061-04 |

| HitHunter Kinase Assay Kit - p34 ^{cdc2} | Code number |
|--|-------------|
| 100 (96 well) | 90-0062-01 |
| 800 (384 well) | 90-0062-02 |
| 10 000 (384 well) | 90-0062-03 |
| 40 000 (384 well) | 90-0062-04 |

| HitHunter Kinase Assay Kit - Kemptide | Code number |
|---------------------------------------|-------------|
| 100 (96 well) | 90-0063-01 |
| 800 (384 well) | 90-0063-02 |
| 10 000 (384 well) | 90-0063-03 |
| 40 000 (384 well) | 90-0063-04 |

| HitHunter Kinase Assay Kit - ATF2 | Code number |
|-----------------------------------|-------------|
| 100 (96 well) | 90-0064-01 |
| 800 (384 well) | 90-0064-02 |
| 10 000 (384 well) | 90-0064-03 |
| 40 000 (384 well) | 90-0064-04 |

| HitHunter Kinase Assay Kit - Crosstide | Code number |
|--|-------------|
| 100 (96 well) | 90-0065-01 |
| 800 (384 well) | 90-0065-02 |
| 10 000 (384 well) | 90-0065-03 |
| 40 000 (384 well) | 90-0065-04 |

| HitHunter Kinase Assay Kit - CHKtide | Code number |
|--------------------------------------|-------------|
| 100 (96 well) | 90-0066-01 |
| 800 (384 well) | 90-0066-02 |
| 10 000 (384 well) | 90-0066-03 |
| 40 000 (384 well) | 90-0066-04 |

| HitHunter Kinase Assay Kit - PTK | Code number |
|----------------------------------|-------------|
| 100 (96 well) | 90-0067-01 |
| 800 (384 well) | 90-0067-02 |
| 10 000 (384 well) | 90-0067-03 |
| 40 000 (384 well) | 90-0067-04 |

| HitHunter Kinase Assay Kit - PKC19-31 | Code number |
|---------------------------------------|-------------|
| 100 (96 well) | 90-0069-01 |
| 800 (384 well) | 90-0069-02 |
| 10 000 (384 well) | 90-0069-03 |
| 40 000 (384 well) | 90-0069-04 |

| HitHunter Kinase Assay Kit - MBP | Code number |
|----------------------------------|-------------|
| 100 (96 well) | 90-0070-01 |
| 800 (384 well) | 90-0070-02 |
| 10 000 (384 well) | 90-0070-03 |
| 40 000 (384 well) | 90-0070-04 |