Calbiochem®

# Protein Kinase Assay & Detection Kits

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**Includes:**

- ELISA Kits
- PhosphoDetect™ ELISA Kits
- K-LISA™ Activity Kits
- TruLight™ Kinase Assays
- Related Products
- Technical Tips

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# Protein Kinase Assay & Detection Kits

Protein kinases are enzymes that phosphorylate target proteins, typically resulting in target activation. Because phosphorylation events regulate myriad signal transduction/disease pathways, kinases are attractive drug targets and the subject of intense research. Over the past 15 years, highly sensitive and specific methods have been created to monitor the level and activity of several kinases. As a result, a large selection of kinase assay and detection kits is available, offering increased flexibility for researching signaling pathways and kinase function. Due to the wide variety of kits, it is important to understand the format, strengths, limitations, and requirements of each assay.

**Enzyme-linked immunosorbent assays (ELISAs)** can be used to quantitate kinase levels and evaluate their activities. ELISAs are popular because they are generally fast, inexpensive, easily accommodate multiple samples, and do not require highly specialized equipment. By incorporating pan (recognize protein regardless of phosphorylation state) and phospho-specific antibodies, ELISA-based assays can be used to measure total protein and activated protein levels. ELISA kits offer an indirect measurement of kinase activity, but because they are antibody-based, they are specific, sensitive, and can be used with a variety of sample types.

Different formats for direct or indirect measurement of kinase activity provide increased flexibility

**Calbiochem® K-LISA™** kits utilize a peptide or polypeptide substrate and phospho-specific antibodies to directly measure kinase activity in a wide variety of samples, including cell lysates and purified or partially purified kinase samples. K-LISA kits combine the ease of use and sensitivity of ELISA kits with direct measurement of kinase activity and can accommodate inhibitor, effector, and immunoprecipitation (IP) based studies (Figures 1 & 2). More information about K-LISA assays can be found on pages 6 & 7 of this brochure.

**Activity assays** utilizing colorimetric or Western blot detection are also available. These kits vary in the sample type, applications, and equipment needed. Refer to the Table on page 6 and read the protocol before choosing.

**TruLight™ Kinase Assays** measure enzyme activity directly without washing steps, in a homogeneous format. Because they can be easily automated, they can be used for high-throughput screening (HTS) applications. TruLight Kinase Assays utilize a proprietary superquenching technology that does not require antibodies or radioactivity. More information on TruLight Kinase Assays can be found on pages 8 & 9 of this brochure.

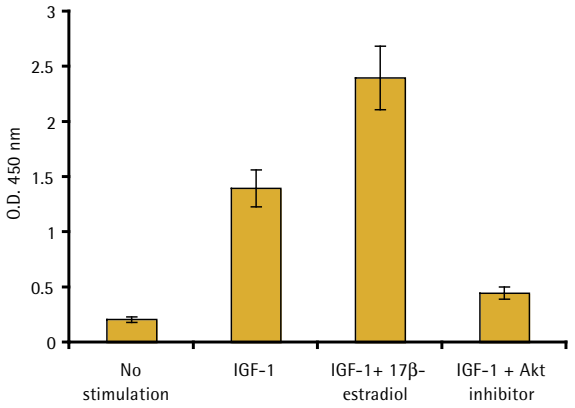



**Figure 1. K-LISA kits measure kinase activity in purified or partially purified samples, and can be used to monitor the effects of activators or inhibitors.**

K-LISA Akt Activity Kit (Cat. No. CBA019) was used to measure immunoprecipitated Akt kinase activity in MCF-7 cells stimulated with IGF-1 and 17β-estradiol. Inhibition of Akt by Akt Inhibitor II (Cat. No. 124008) following stimulation with IGF-1 is also demonstrated.

Having a wide variety of kinase assay and detection kits is important because different stages of kinase research have different requirements. In the early discovery phases of kinase research, the specificity that an antibody-based technique such as ELISA provides is needed. In the later analysis stages, higher speed is required, and peptide-based methods are more suitable. The methods can be complementary to each other in a protein kinase research project, and can also utilize the same Calbiochem® companion products such as inhibitors, substrates, and enzymes. Use this brochure and the information on our website to determine which kits and products will meet your needs.

## How to choose a protein kinase kit

When choosing a kinase kit, first consider your goal and determine which measurements would serve the purpose. For example, if you would like to measure active Akt levels, you could measure the amount of phosphorylated Akt using a PhosphoDetect™ ELISA kit or you could measure Akt activity directly using a K-LISA™ Activity Kit. If a kit is not available for your protein kinase of interest, consider measuring the activity indirectly by measuring the phosphorylation of one of its targets using a PhosphoDetect ELISA kit or antibody.

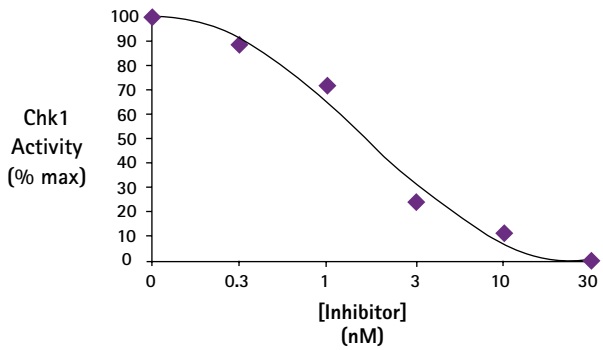



**Figure 2. Staurosporine inhibition of Chk1 measured by K-LISA Checkpoint Activity Kit (Cat. No. CBA020).**

The activity of Chk1, His•Tag®, Human, Recombinant (Cat. No. 220479) (1.1 ng) was determined in the presence of increasing Staurosporine (Cat. No. 569397) concentration.

Next, consider the sample available for testing. Cell extracts contain a plethora of kinases, so using an immunoprecipitating (IP) antibody approach or a sandwich-type PhosphoDetect ELISA kit would be most preferable since activity kits may contain a nonexclusive substrate. Functional studies with purified enzymes are obviously best suited for activity assays such as K-LISA or TruLight because they directly measure kinase activity and allow for attenuation by an inhibitor or stimulation by an activator. If you need to screen many kinases or inhibitors, choose a TruLight Kinase Assay, because they do not require mixing steps, making them easier to use and fully automatable.

An additional consideration is the mechanism of the inhibitor/activator utilized. For example, most specific Akt inhibitors do not directly affect the activity of an enzyme, but affect its regulation, so they would not be suitable for use with a kit that directly measures Akt activity. A more general, direct-acting inhibitor such as Staurosporine (Cat. No. 569397) should be used with a kit such as the K-LISA Akt Activity Kit. Check the sensitivity and range of the assay to ensure that it matches the expected level of kinase in your sample. Finally, make sure that the species reactivity is applicable for your sample. In general, species reactivity will be broader for activity assays than for ELISA kits. However, any step involving antibody detection (e.g., immunoprecipitation) may limit the species reactivity of an activity assay to the species reactivity of the antibody. Refer to the kinase kit selection guide table on the bottom of this page for general guidelines and additional information.

## More information can be found on the web at www.calbiochem.com/kinasekits

| Protein Kinase Kit Selection Guide | | ELISA | PhosphoDetect ELISA | K-LISA | Activity Assay | TruLight |
|---|---|---|---|---|---|---|
| Sample Type | Tissue homogenates | ✓ | ✓ | | | |
| | Cell lysates | ✓ | ✓ | ✓ | ✓ | |
| | Partially purified | ✓ | ✓ | ✓ | ✓ | |
| | Purified enzyme | | | ✓ | ✓ | ✓ |
| Inhibitor Type | Upstream inhibitors | ✓ | ✓ | | | |
| | Direct inhibitors | | | ✓ | ✓ | ✓ |
| Applications | Inhibitor screening | | | ✓ | ✓ | ✓ |
| | High-throughput screening | | | | | ✓ |

# Kinase ELISAs and PhosphoDetect™ ELISAs

## For detecting total and phosphorylated protein kinase levels

### Summary

Calbiochem® Protein Kinase ELISAs (Enzyme-linked immunosorbent assays) measure total or phosphorylated kinase levels in biological samples, see example data in Figure 3. ELISA kits come with 96-well plates which have been coated with antibodies against the kinase to be detected (Figure 4). When the sample is added, the kinase binds to the antibody, and the plate is washed. A solution containing a detector antibody, also specific for the kinase, is added, which binds the bound kinase. For PhosphoDetect ELISAs, the detector antibody is phospho-specific, recognizing only the phosphorylated form of the protein. After an additional washing step, a secondary antibody which will recognize the bound antibody is added. This antibody is also conjugated with horseradish peroxidase (HRP), and after an additional washing step, the quantity of the protein can be detected colorimetrically by the addition of a substrate for HRP.

### When to use

Use these kits to quantitate levels of total or phosphorylated protein kinase

### Advantages

- Specific, sensitive detection of kinase levels in non-purified samples
- Detection can be species-specific
- No special equipment required

### Considerations

- Does not measure kinase activity directly
- Direct inhibitors/activators cannot be used

### Sample Type

Tissue homogenates, cell extracts, partially purified protein kinase samples

### Detection

Colorimetric

### Equipment Required

Colorimetric ELISA plate reader

Protein Kinase ELISA Kits

| Kit Name | Cat. No. | # Tests | 96-well format | Species | Assay range | Positive Control |
|---|---|---|---|---|---|---|
| c-ErbB2/c-Neu Rapid Format ELISA Kit | QIA10 | 96 | Y | human | 0.024-3 ng/ml | c-erbB2/c-neu |
| Epidermal Growth Factor Receptor (EGF-R) ELISA | CBA018 | 96 | Y | human | 0.062-2 ng/ml | EGF-R |
| ERK 1/2 ELISA Kit | CBA032 | 96 | Y | human, mouse, rat | 0.03-2 ng/ml | ERK 1/2 |
| Insulin Receptor (β-subunit) ELISA Kit | CBA039 | 96 | Y | human, mouse, rat | 0.94-60 ng/ml | Insulin Receptor (β-subunit) |
| JNK 1/2 ELISA Kit | CBA033 | 96 | Y | human, mouse | 0.31-20 ng/ml | JNK 1/2 |
| c-Met ELISA Kit | CBA031 | 96 | | human | 0.78-50 ng/ml | c-Met |
| p38 MAP Kinase ELISA Kit | CBA029 | 96 | Y | human, mouse, monkey | 31.2-2000 pg/ml | p38 MAP Kinase |
| Src ELISA Kit | CBA027 | 96 | Y | human, mouse, rat | 1.6-50 ng/ml | Src |

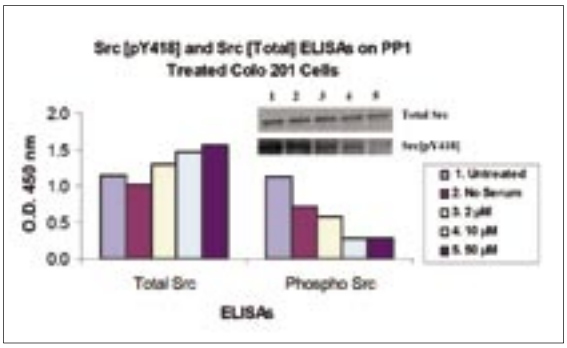



**Figure 3. Src ELISA Kit (Cat. No. CBA027) and PhosphoDetect Src ($pTyr^{418}$) ELISA Kit (Cat. No. CBA028) kits used in conjunction to monitor kinase activation.**

Treatment with PP1, a specific inhibitor of Src kinase, inhibited phosphorylation of Src in Colo 201 cells in a dose-dependent manner. The amount of total Src remained comparable, while the level of Src phosphorylated at tyrosine 418 decreased with increasing doses of PP1.

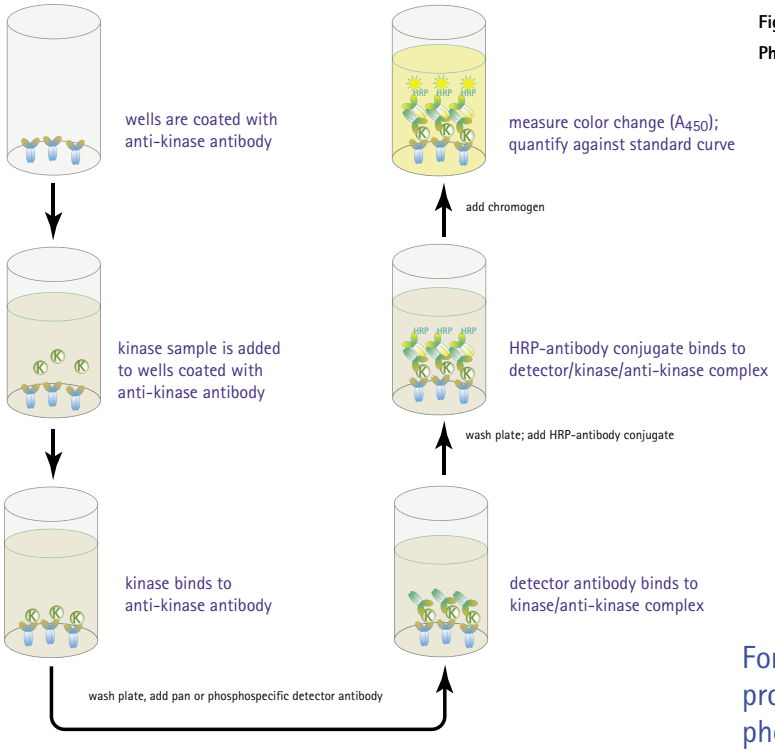



**Figure 4. Calbiochem® Kinase ELISA and PhosphoDetect ELISA Procedure.**

For a complete listing of PhosphoDetect products, including over 200 phospho-specific antibodies, visit www.calbiochem.com/phosphodetect

## PhosphoDetect™ Protein Kinase ELISA Kits

| Kit Name | Cat. No. | # Tests | 96-well format | Species | Assay range* | Positive Control |
|---|---|---|---|---|---|---|
| PhosphoDetect Akt ($pSer^{473}$) ELISA Kit | CBA005 | 96 | Y | human, mouse, rat | 1.6-100 Units/ml | $pSer^{473}$ Akt |
| PhosphoDetect Akt ($pThr^{308}$) ELISA Kit | CBA004 | 96 | Y | human, mouse, rat | 1.6-100 Units/ml | $pThr^{308}$ Akt |
| PhosphoDetect EGF Receptor ELISA Kit | QIA95 | 96 | Y | human | 0.1-5 fmol/ml | EGF-R |
| PhosphoDetect ERK 1/2 ($pThr^{185}$/$pTyr^{187}$) ELISA Kit | CBA006 | 96 | Y | human, mouse, rat | 1.6-100 Units/ml | $pThr^{185}$/$pTyr^{187}$ ERK 1/2 |
| PhosphoDetect Insulin Receptor ($pTyr^{1162/1163}$) ELISA Kit | CBA038 | 96 | Y | human, mouse, rat | 1.6-100 Units/ml | $pTyr^{1162/1163}$ Insulin Receptor |
| PhosphoDetect JNK1/2 ($pThr^{183}$/$pTyr^{185}$) ELISA Kit | CBA007 | 96 | Y | human | 1.6-100 Units/ml | $pThr^{183}$/$pTyr^{185}$ JNK1/2 |
| PhosphoDetect MEK1 ($pSer^{218/222}$) ELISA Kit | CBA030 | 96 | Y | human, mouse | 1.6-100 Units/ml | $pSer^{218/222}$ MEK1 |
| PhosphoDetect p38 MAP Kinase ($pThr^{180}$/$pTyr^{182}$) ELISA Kit | CBA008 | 96 | Y | human, mouse | 1.6-100 Units/ml | $pThr^{180}$/$pTyr^{182}$ p38 MAP Kinase |
| PhosphoDetect Src ($pTyr^{418}$) ELISA Kit | CBA028 | 96 | Y | human, mouse, rat | 1.6-100 Units/ml | $pTyr^{418}$ Src |

*Unit definition varies; see individual protocols for more information.

# K-LISA™ and Activity Assays

## For direct measurement of protein kinase activity

### Summary

Calbiochem® offers kits with several different formats for the direct measurement of kinase activity. K-LISA kits measure kinase activity utilizing a technique in which phosphorylation of a target is detected using phospho-specific antibodies (see Figure 5). Kits utilizing Western blots (Cat. No. 124007 and 420115) offer a straightforward method for measuring kinase activity through detection of phosphorylated protein substrates.

### When to use

Use these kits to measure protein kinase activity and determine the effects of activators or inhibitors. The kits vary in sample compatibility, so this should be considered carefully when choosing a kinase activity kit.

### Advantages

- Measure kinase activity directly
- Direct effects of inhibitors/activators can be measured

### Considerations

Cell lysates or partially purified samples should be used with caution because peptide substrates can often be phosphorylated by multiple kinases.

### Sample Type

Varies – see table below

### Detection

Varies – see table below

### Equipment Required

Colorimetric ELISA plate reader or Western blotting equipment

K-LISA Kits

| Kit Name | Cat. No. | # Tests | Detection | 96-well format | Sample Type | Assay range or lower limit of detection (LOD) |
|---|---|---|---|---|---|---|
| K-LISA Akt Activity Kit | CBA019 | 96 | Colorimetric | Y | Cell lysates, partially purified or purified enzyme | Assay Range = 6-100 mU*/well |
| K-LISA Checkpoint Activity Kit | CBA020 | 96 | Colorimetric | Y | Cell lysates, partially purified or purified enzyme | Assay Range = Chk1: 25-725 µU, Chk2:260 µU – 26 mU |
| K-LISA mTOR Activity Kit | CBA055 | 96 | Colorimetric | Y | Cell lysates | |
| K-LISA IKKβ Inhibitor Screening Kit | CBA044 | 96 | Colorimetric | Y | Cell lysates, partially purified or purified enzyme | Assay Range = 21-840 mU |
| K-LISA PTK Screening Kit | 539701 | 144 | Colorimetric | Y | Cell lysates, partially purified or purified enzyme | Assay Range = µU (e.g., FLT3, c-Abl, βInsR) to mU (e.g., Src, EGFR) |

*Unit is defined as the amount of enzyme that will transfer 1 nmol phosphate to substrate per min at 30°C.

Activity Assay Kits

| Kit Name | Cat. No. | # Tests | Detection | 96-well format | Sample Type | Species |
|---|---|---|---|---|---|---|
| Akt Activity Immunoassay Kit | 124007 | 40 | Western blot | N | tissue homogenates, cell extracts | human, mouse, rat |
| JNK Activity Immunoassay Kit | 420115 | 40 | Western blot | N | cell lysates | human, mouse, rat |
| Protein Kinase Assay Kit, Non-radioactive | 539484 | 96 | Colorimetric | Y | cell lysates, purified enzyme | broad |

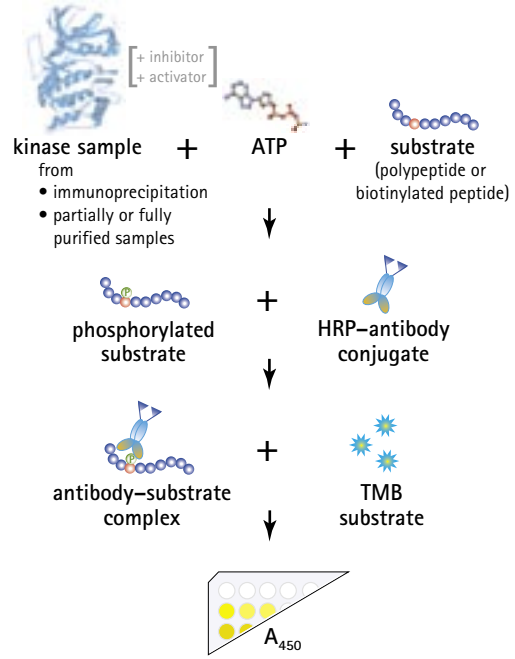



**Figure 5. K-LISA™ Schematic.**

The sample containing the protein kinase to be assayed is incubated with ATP and the polypeptide or peptide substrate. The K-LISA Checkpoint Activity Kit (Cat. No. CBA020) uses a biotinylated peptide substrate phosphorylated by Chk1 and Chk2. Similarly, the K-LISA Akt Activity Kit (Cat. No. CBA019) uses a peptide substrate phosphorylated by Akt1, Akt2, and Akt3. The K-LISA PTK Screening Kit (Cat. No. 539701) uses three different random polypeptide substrates (EAY, $E_4Y$, or EY) composed of different molar ratios of glutamic acid (E), alanine (A), and tyrosine (Y). An HRP-antibody conjugate is added (in some kits a detection antibody is also required), and this is followed by addition of the TMB substrate and detection at 450 nm.

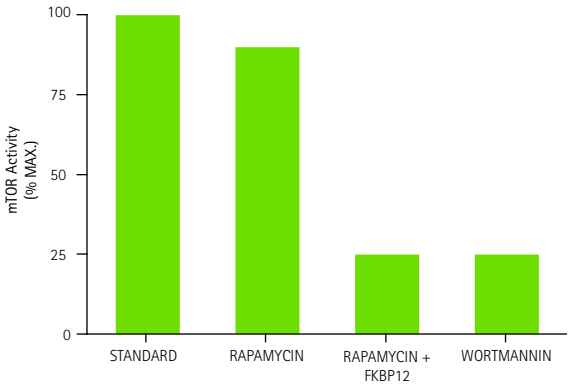



**Figure 6. Rapamycin + FKBP12 Inhibition of mTOR Kinase Activity measured using K-LISA mTOR Activity Kit (Cat. No. CBA055).**

FKBP12 + Rapamycin inhibition of mTOR Standard. The activity of the mTOR Standard, (50 µl) was determined in the presence of either Rapamycin (20 µM; Cat. No. 553210 or 553211), Rapamycin (20 µM) + GST-FKBP12 (37 µg/ml), or Wortmannin (10 µM; Cat. No. 681675; also included with the kit). Addition of Wortmannin serves as a positive control for inhibitor analysis. Inhibition profiles can be generated based on mTOR activity in the presence and absence of test inhibitor(s).

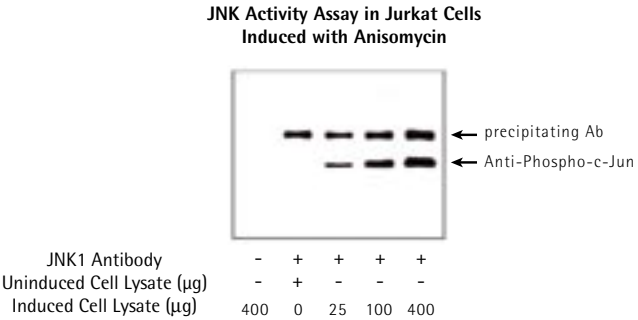



**Figure 7. Detection of JNK activity with JNK Activity Immunoassay Kit (Cat. No. 420115).**

The assay utilizes a JNK-specific antibody to immunoprecipitate JNK from cell lysates, after which JNK activity is detected in a kinase reaction using c-Jun as a substrate. Phosphorylation of c-Jun can be analyzed by Western blot using a c-Jun phospho-specific antibody. An increase in JNK in Jurkat Cells following anisomycin treatment is shown. NOTE: The heavy chain of the precipitating antibody is detected by the secondary antibody.

# TruLight™ Kinase Assays

## For fast, sensitive measurement of protein kinase activity

### Summary

TruLight Kinase Assays measure activity in recombinant or purified kinases by detecting phosphorylation of a peptide substrate (Figure 8). The format is homogeneous (does not require washing steps), so the assays are fast and can be automated. The assays utilize a proprietary fluorescence superquenching technology which results in assays that are highly sensitive (Figures 9 and 10). As a result, higher Z' values can be obtained with less enzyme, making the assays more cost effective (Figure 11). Eleven ready-to-use kits are available, or develop your own using the TruLight Universal Kinase/Phosphatase Assay Kit (Cat. No. 539714).

### When to use

Use these kits to measure kinase activity directly and to measure the effects of activators or inhibitors for recombinant or purified kinases. The assays are homogeneous and can be automated.

### Advantages

- Homogeneous format
- No radioactivity or antibodies
- Up to 10 times more sensitive than FRET-based assays
- Excellent Z' values ( $\geq$ 0.7 at 10% conversion)
- High ATP tolerance ( $\geq$ 100 µM)
- No custom equipment; standard fluorescence intensity measurement

### Considerations

Cell lysates or partially purified samples should be used with caution as peptide substrates can often be phosphorylated by multiple kinases

### Sample Type

Recombinant or purified enzymes

### Detection

Fluorescent intensity

### Equipment Required

Fluorescent plate reader (excitation wavelength = 450 nm, emission wavelength = 490 nm, cut-off = 475 nm)

For more information
visit www.calbiochem.com/trulight

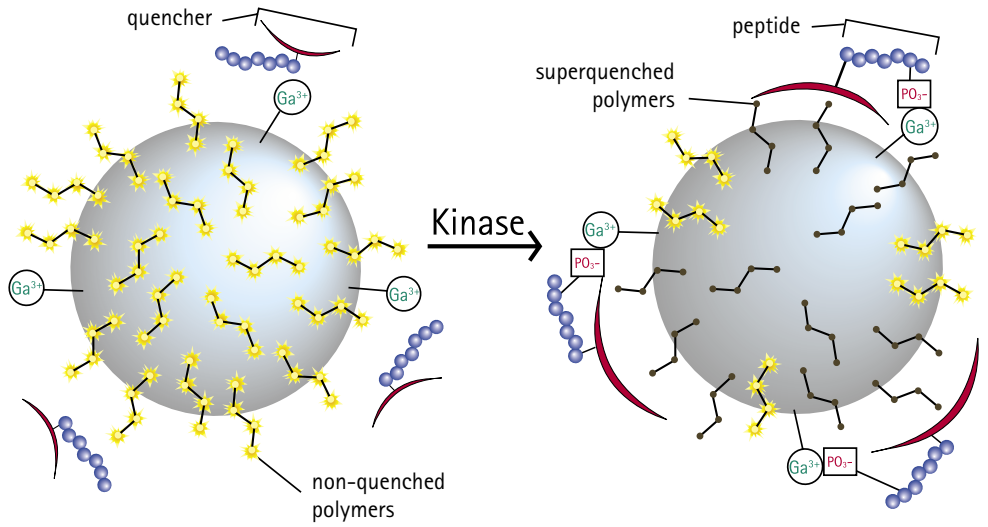
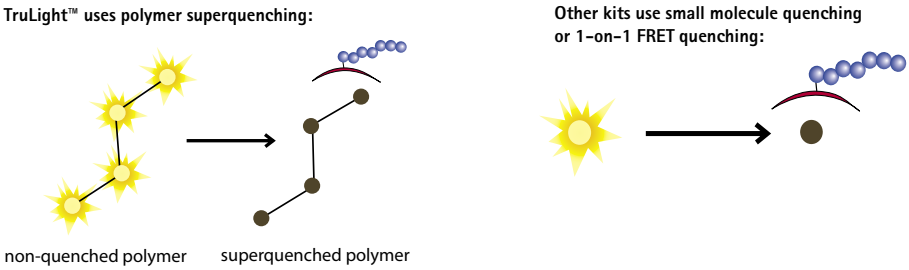


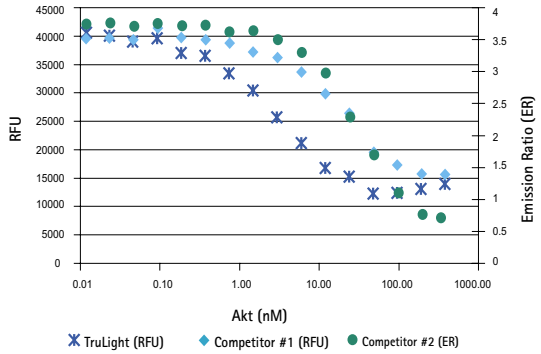

**Figure 8.**

TruLight Kinase Assay Kits employ a proprietary superquenching technology utilizing microspheres that are coated with fluorescent polymers and a metal ion coordination complex. Peptide substrates are labeled with a quencher that will quench fluorescence when bound. When the peptide becomes phosphorylated, it binds the microsphere, resulting in fluorescence quenching and a decrease in the fluorescent signal.



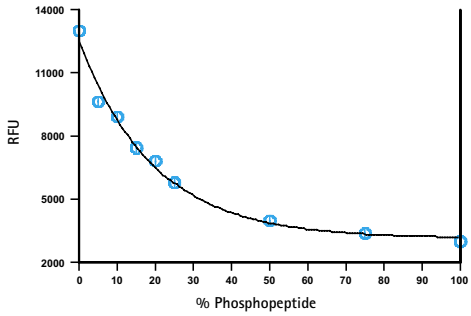

## Figure 9: The Advantage of Superquenching

Unlike fluorescence resonance energy transfer (FRET) assays or traditional fluorescence quenching assays, where the quench is an equimolar event between donor and acceptor, the TruLight assay exploits the phenomenon of superquenching, resulting in a 50-fold increase in quenching power. As a result, TruLight assays are up to 10 times more sensitive than FRET-based assays, allowing you to use less enzyme in your screening assay, while achieving higher Z' values at lower enzyme conversion.




## Figure 10: TruLight Kinase Assay Kits Are More Sensitive Than Other FRET-based Assays.

TruLight Akt1/PKBα Kinase Assay Kit (Cat. No. 539705) was tested against two competitors. ATP concentrations and reaction conditions were according to each protocol. TruLight is 2-fold more sensitive than competitor #1 and 6-fold more sensitive than competitor #2, based on $EC_{50}$ values.




## Figure 11: TruLight Kinase Assay Kits Are Robust, Yielding High Signal to Noise Ratios at Low Enzyme Conversion.

Change in Relative Fluorescence Unit (RFU) signal is shown versus % phosphopeptide for the TruLight Src Kinase Assay Kit (Cat. No. 539706). TruLight Assay Kits are optimized for maximal signal change/higher Z' values at less than 25% conversion. Thus, TruLight kits provide more meaningful data with less enzyme, decreasing your costs without compromising on data integrity.

## TruLight Kinase Assays

| Kit Name | Cat. No. | # Tests | 96-well format | Lower limit of detection (LOD) | Positive Control |
|---|---|---|---|---|---|
| TruLight Akt1/PKBα Kinase Assay Kit | 539705 | 96 | Y | 25 pM | GRPRTSpSFAEG |
| TruLight Aurora A Kinase Assay Kit | 539716 | 96 | Y | 6.9 pM | LRRApSLG |
| TruLight ERK1/2 Assay Kit | 539715 | 96 | Y | ERK1 25 pM, ERK2 66 pM | N/A |
| TruLight p38α Kinase Assay Kit | 539710 | 96 | Y | 211 pM | N/A |
| TruLight p70S6 Kinase Assay Kit | 539711 | 96 | Y | 800 pM | GRPRTSpSFAEG |
| TruLight PKA Assay Kit | 539712 | 96 | Y | 1.2 pM | LRRApSLG |
| TruLight $PKC_{\alpha}$ Assay Kit | 539707 | 96 | Y | 2.5 pM | RFARKGpSLRQKNV |
| TruLight $PKC_{\beta I/II}$ Assay Kit | 539713 | 96 | Y | $PKC_{\beta I}$ 4.2 pM, $PKC_{\beta II}$ 2.2 pM | RFARKGpSLRQKNV |
| TruLight $PKC_{\varepsilon}$ Assay Kit | 539708 | 96 | Y | 7.6 pM | RFARKGpSLRQKNV |
| TruLight RSK-2 Assay Kit | 539709 | 96 | Y | 75 pM | GRPRTSpSFAEG |
| TruLight Src Kinase Assay Kit | 539706 | 96 | Y | 31.6 pM | AAAEEIpYGEI |
| TruLight Universal Kinase/ Phosphatase Assay Kit | 539714 | 400 | Y | Varies | (user provides) |

# Related Products: Kinase Inhibitor Sets

| Product | Cat. No. | Comments | Size |
|---|---|---|---|
| MAP Kinase Cascade Inhibitor Set | **444185** | A set of 5 vials. Each set contains 1 mg each of FPT Inhibitor III (Cat. No. 344154) and ZM 336372 (Cat. No. 692000), 5 mg of PD 98059 (Cat. No. 513000) and 1 mg of SB 203580 (Cat. No. 559389). | 1 set |
| MAP Kinase Inhibitor Set I | **444180** | A set of 4 vials. Each set contains 5 mg of the MEK inhibitor PD 98059 (Cat. No. 513000), 1 mg each of the MAP kinase inhibitors SB 202190 (Cat. No. 559388) and SB 203580 (Cat. No. 559389), and 1 mg of the negative control, SB 202474 (Cat. No. 559387). Supplied with a directional insert. | 1 set |
| MAP Kinase Inhibitor Set II | **444190** | A set of 4 vials. Each set contains 5 mg of PD 98059 (Cat. No. 513000), 1 mg each of SB 203580 (Cat. No. 559389), and U0126 (Cat. No. 662005), and 1 mg of the negative control, SB 202474 (Cat. No. 559387). | 1 set |
| Protein Kinase C Inhibitor Set | **539573** | A set of 6 vials. Each set contains 250 µg of Bisindolylmaleimide I (Cat. No. 203290), 50 µg of Calphostin C, *Cladosporium cladosporioides* (Cat. No. 208725), 5 mg of Chelerythrine Chloride (Cat. No. 220285), 500 µg of Gö 6976 (Cat. No. 365250), 500 µg of Myristoylated Protein Kinase C Inhibitor 20-28, Cell-Permeable (Cat. No. 476480), and 500 µg of Ro-32-0432 (Cat. No. 557525). | 1 set |
| Protein Kinase Inhibitor Set | **476490** | A set of 6 vials. Each set contains 500 µg of Ro-31-8220 (Cat. No. 557520), 20 mg of Genistein (Cat. No. 345834), 1 mg of KN-93, Water-Soluble (Cat. No. 422711), 50 µg of KT5720 (Cat. No. 420320), 100 µg of Staurosporine, *Streptomyces* sp. (Cat. No. 569397), and 1 mg of U0126 (Cat. No. 662005). | 1 set |
| Serine/Threonine Kinase Inhibitor Set | **539572** | A set of 6 vials. Each set contains 250 µg of PKC inhibitor, Bisindolylmaleimide I (Cat. No. 203290); 1 mg of PKA inhibitor, H-89, Dihydrochloride (Cat. No. 371963); 1 mg of PKG inhibitor, Protein Kinase G Inhibitor (Cat. No. 370654); 1 mg of MLCK inhibitor, ML-7 (Cat. No. 475880); 1 mg of CaM kinase II inhibitor, KN-93 (Cat. No. 422708); and 100 µg of the broad range inhibitor, Staurosporine (Cat. No. 569397). Not available for sale in Japan. | 1 set |
| Src Family Protein Tyrosine Kinase Inhibitor Set | **567816** | A set of 4 vials. Each set contains 20 mg of Genistein (Cat. No. 345834), 100 µg of Herbimycin A, *Streptomyces* sp. (Cat. No. 375670), and 1 mg each of PP2 (Cat. No. 529573) and PP3 (Cat. No. 529574). | 1 set |
| Tyrosine Kinase Inhibitor Set II | **657021** | A set of 4 vials. Each set contains 20 mg of Genistein (Cat. No. 345834), 1 mg of PP2 (Cat. No. 529573), 5 mg of AG 490 (Cat. No. 658401), 5 mg of AG 1296 (Cat. No. 658551), and 5 mg of AG 1478 (Cat. No. 658552). | 1 set |

NEW

# Online Inhibitor Resource

## www.calbiochem.com/inhibitor

New! Online Inhibitor Resource, allowing you to browse over 1200 inhibitors for phosphorylation, apoptosis/ cancer, lipid signaling, neurobiology/neurodegeneration research, and more. Keep up with the newest inhibitors for your research!

# Technical Tips

## What's the best way to get consistent results when using a kit?

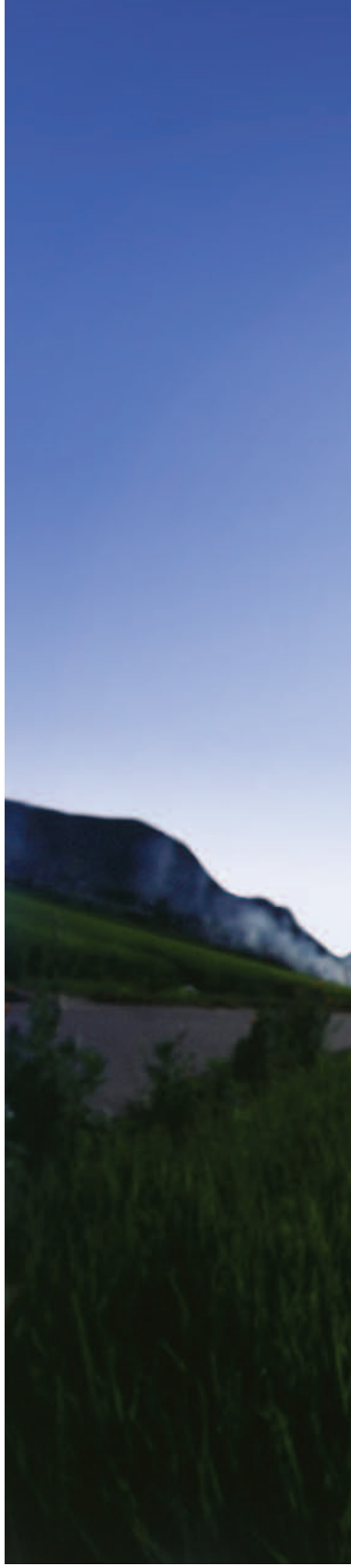
- Always use components from the same lot of a given kit, including the standard, since each lot contains kit components that are optimized to work together.
- When applicable, perform a standard curve with each new experiment.
- Use externally prepared standards (e.g., purified enzymes, cell lysates) only as experimental controls; do not replace the included standards with standards from another source.

## What types of samples can be used?

- Different kit protocols are optimized for use with different sample types, so it is recommended that this information be confirmed in the protocol. Depending on the enzyme, the level of detectable activity can vary among sample types. It is also important to note any species reactivity limitations.
- Using subcellular fractions (*e.g.,* nuclear or membrane fractions) may improve target detection. Try the ProteoExtract® Subcellular Proteome Extraction Kit (Cat. No. 539790) to prepare four separate fractions from one sample: cytoskeletal, nuclear, membrane/organelle, and cytosolic.
- For optimizing detection of phosphorylation, use phosphatase inhibitors (see www.calbiochem.com/inhibitor for complete list of products) or PhosphoSafe™ Extraction Reagent (Cat. No. 71296), an enhancement of the CytoBuster™ Protein Extraction reagent, with four phosphatase inhibitors, to prepare your sample.

## How do I choose a protein kinase inhibitor for my assay?

- Consider which protein kinase must be inhibited to achieve the experimental results desired. For a direct kinase assay such as K-LISA, inhibit the kinase being assayed, but for PhosphoDetect™ ELISA kits, choose inhibitors that affect kinase(s) upstream of the target.
- Consider cell permeability, mode of action, specificity, and $K_i/IC_{50}$ values for each inhibitor. Refer to the Calbiochem® Inhibitor Sourcebook™ or our online inhibitor resource, www.calbiochem.com/inhibitor, to find this information. Refer also to two excellent reviews on kinase inhibitor specificities: Davies, S. P., et al. 2000. *Biochem. J.* **351**, 95; Bain, J., et al. 2003. *Biochem. J.* **371**, 199.
- In general, samples containing protein kinases of interest should be pre-incubated with an inhibitor for 15-30 min prior to performing the activity assay.



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Protein Kinase Assay & Detection Kits

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