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## Mercury™ In Vitro Kinase Assay Kits

Simple, straightforward *in vitro* assay for IKKb or MAPK activity

- Proven method for assaying kinase activity
- Convenient, easy-to-use kit format
- High-quality antibodies included
- IKKb Antibody also available separately

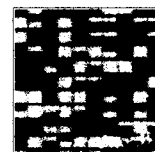
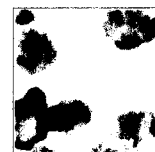
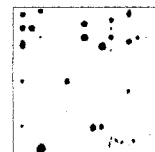
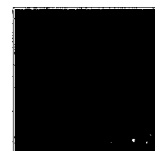
With the new **Mercury™ In Vitro Kinase Assay Kits†**, you can quickly assay for either IKKb or MAPK activity. The *in vitro* kinase assay is simple and straightforward—no time-consuming transfections are required. Each kit contains a highly specific antibody and purified substrate for either IKKb or MAPK; these high-quality reagents result in strong, specific signals with low background. The **IKKb Antibody** is also available separately.

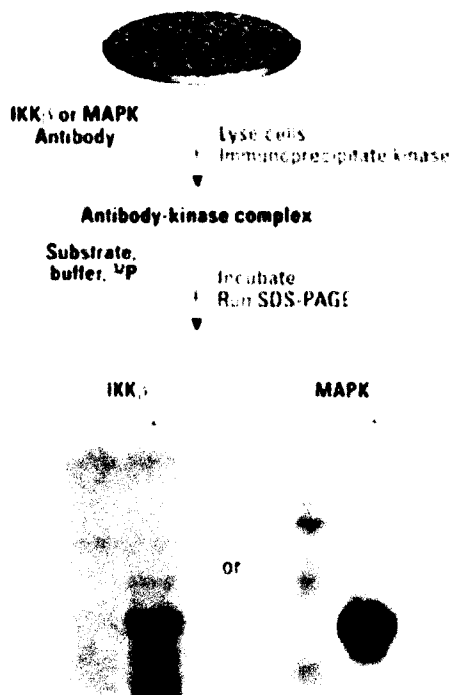
Simple, straightforward *in vitro* assay

Figure 1 illustrates how easy it is to perform the Mercury *in vitro* kinase assay. First, lyse your cells and immunoprecipitate the kinase using the antibody provided in the kit. Then, add the kinase buffer, [ $\gamma$ - $^{32}$ P]dATP, and either your protein of interest or the purified kinase substrate provided. Finally, analyze by SDS-PAGE and autoradiography. A band appears on the X-ray film if the kinase phosphorylates the substrate.

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**Figure 1. The simple Mercury™ *In Vitro* Kinase Assay. IKKb data.** Untreated (–) and TNF-treated (+) HeLa cell extracts were analyzed using the IKKb *In Vitro* Kinase Assay Kit. Cell lysates were immunoprecipitated with the IKKb Antibody and incubated with the IKKb Substrate (IkB). Phosphorylation was measured using [ $^{32}\text{P}$ ]dATP. **MAPK data.** Untreated (–) and serum-treated (+) 3T3 cell extracts were analyzed using the MAPK *In Vitro* Kinase Assay Kit. Cell lysates were immunoprecipitated with the MAPK Antibody and incubated with the MAPK Substrate (Elk-1). Phosphorylation was measured using [ $^{32}\text{P}$ ]dATP.

The Mercury *in vitro* kinase assay has many potential applications. For example, you can screen cells that have been treated with drugs, environmental toxins, or proteins to see if IKKb or MAPK is activated; in this case, you would use the purified kinase substrate provided in the kit. Alternatively, you can use your protein of interest as the substrate to see if it is phosphorylated by IKKb or MAPK. In any application, the Mercury *in vitro* kinase assay quickly provides information about the activity of IKKb or MAPK.

#### Investigate IKKb or MAPK activity *in vitro*

TOP

IKKb was recently identified as a key kinase in the NF $\kappa$ B pathway (Figure 3; see reference 1 for review), an essential component of the basic immune response in mammals. When IKKb phosphorylates IkB, IkB dissociates from NF $\kappa$ B and is degraded, causing activation of NF $\kappa$ B. The NF $\kappa$ B pathway is known to be induced by stimulus of the TNF or IL-1 lymphokine receptors; however, identifying new agonists and antagonists could potentially lead to new therapies for cancers and immunological disorders. CLONTECH offers a number of [other Mercury products](#) for studying the NF $\kappa$ B pathway, including several Mercury Pathway Profiling Vectors and the IkB-EGFP Signaling Probe.

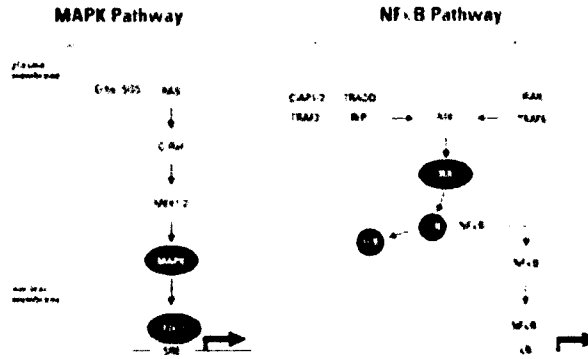


Figure 3. The NFκB and MAPK pathways.

[Larger Image of Figure 3](#)

The MAPK pathway is a crucial signaling pathway implicated in cell proliferation (Figure 3). MAPK, also known as ERK1/2, is induced by a number of mitogenic stimuli. Activated MAPK phosphorylates the transcription factor Elk-1, which activates genes involved in cell proliferation and differentiation. CLONTECH's MAPK *In Vivo* Kinase Assay Kit is a complementary product for MAPK studies.

Superior antibodies—high specificity, low background 10P  
 The key to our *In Vitro* Kinase Assay Kits is our high-quality antibodies. The Western blots shown in Figure 2 demonstrate the strong, specific signal and low background levels that are typical of the IKKβ and MAPK antibodies.

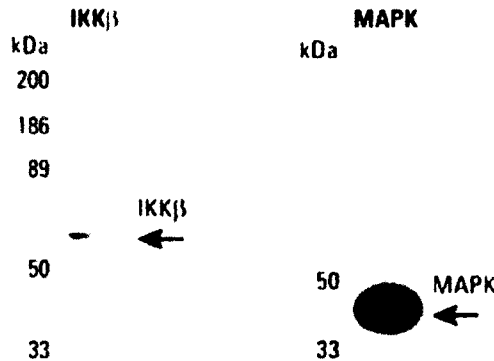


Figure 2. The IKKβ and MAPK antibodies exhibit high specificity and low background. **IKKβ data.** Western analysis of the HeLa cell lysate using the IKKβ Peptide Antibody. **MAPK data.** Western analysis of 3T3 cell lysate using the MAPK Peptide Antibody.

**Mercury™—a complete platform of signal transduction products**

Our new Mercury™ *In Vitro* Kinase Assay Kits are the most recent addition to the Mercury product line for studying signal transduction. These new kits are our first products for performing

*in vitro* screening, a quick way to assess kinase activity. Our other products allow you to easily perform *in vivo* studies:

**Mercury™ Pathway Profiling Systems**

Pathway profiling is the first step in determining the role a target gene plays in different signal transduction pathways. The Mercury Pathway Profiling Systems allow you to survey the activation of *cis*-acting enhancer elements *in vivo*.

**Mercury™ In Vivo Kinase Assay Kits**

Once you identify which *cis*-acting enhancer elements are affected by a drug candidate or expression of your target gene, the Mercury *In Vivo* Kinase Assay Kits can help you narrow your focus. Using the *In Vivo* Kinase Assay Kits, you can quickly determine if your target gene or drug candidate induces an increase in kinase activity in a specific signaling pathway.

**Mercury™ EGFP Signaling Probes**

EGFP Signaling Probes provide another way to study your target gene's effects on signaling pathways. Using these probes, you can actually visualize the effects of a gene or stimulus on living cells in real time.

**Mercury™ pCMV-p53 and pCMV-p53mt135 Vector Set**

With these vectors, you can link your target gene to p53 and related signaling pathways. The p53 protein plays an important role in apoptosis, cell growth, and DNA damage repair.

For more information about these products for *in vivo* studies, see reference 2.

Product	Size	Cat. #	
IKKb <i>In Vitro</i> Kinase Assay Kit	40 rxns	K6010-1	New!
MAPK <i>In Vitro</i> Kinase Assay Kit	40 rxns	K6011-1	Discontinued
IKKb Peptide Antibody	100 µg	3810-1	New!

IKKb *In Vitro* Kinase Assay Kit Components

- **IKKb Peptide Antibody**
- **IKKb Substrate (IkB)**
- ***In Vitro* Kinase Buffer**
- **Protein L Agarose Beads**
- **Cell Lysis Buffer**
- **Complete User Manual (PT3406-1)**

MAPK *In Vitro* Kinase Assay Kit Components

- **MAPK Peptide Antibody**
- **MAPK Substrate (Elk-1)**
- ***In Vitro* Kinase Buffer**
- **Protein L Agarose Beads**
- **Cell Lysis Buffer**
- **Complete User Manual (PT3406-1)**

### Related Products

- [Mercury™ Pathway Profiling Systems & Vectors](#) (many)
- [Mercury™ \*In Vivo\* Kinase Assay Kits](#) (many)
- [Mercury™ EGFP Signaling Probes](#) (many)
- [Mercury™ pCMV-p53 & pCMV-p53mt135 Vector Set](#) (#K6004-1)

### References

1. Stancovski, I. & Baltimore, D. (1997) *Cell* 91: 299-302.
2. [Mercury Signal Transduction Products](#) (July 1999)  
*CLONTECHniques* **XIV**(3):16-24.

† Patent pending



This article is also available in Adobe Acrobat (PDF) format.

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