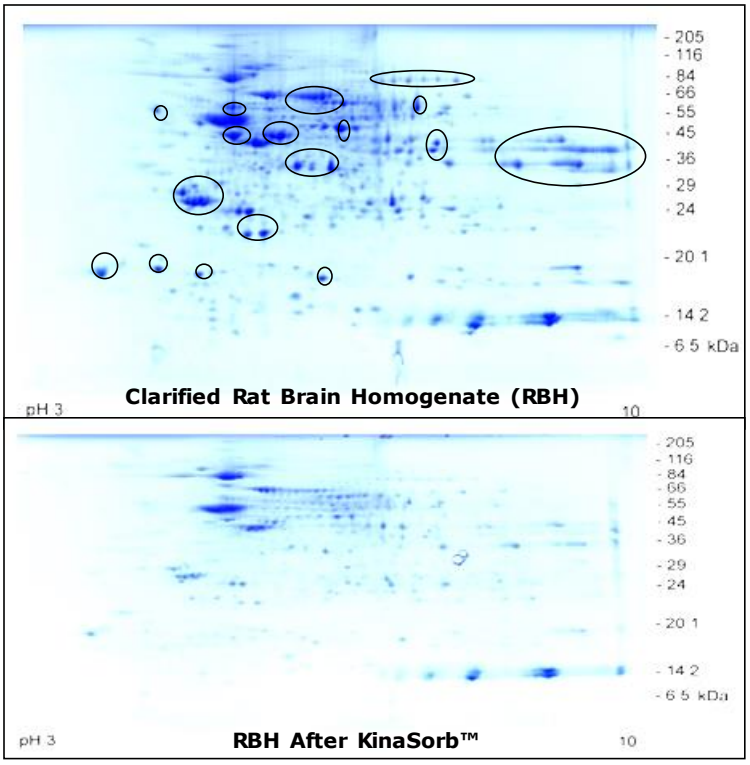
KinaSorb™

Kinase (& ATP binding proteins) enrichment reagent

- Non-covalent immobilization of phosphate group with optimal nucleotide orientation & specificity
- Enrichment 3-5X Kinase enrichment*, protein recoverable, ~200 µg
- 60 minute, scaleable protocol compatible with functional assays, electrophoresis and LC-MS
- Phosphatase activity & cyclic nucleotide phosphodiesterase (PDE) activity not detectable
- Improves protein normalization when comparing heterogeneous tissues
- On-bead digestion protocols for LC-MS annotation

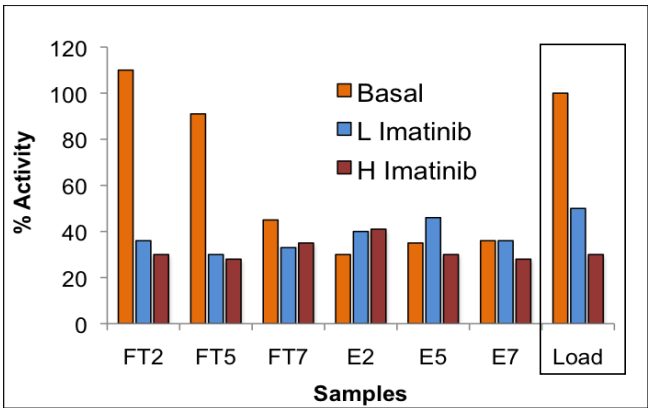
While inhibition of kinases has had clinical success, a major challenge is the sequence conservation of the catalytic domain. Thus, new methods that can prospect into the structure and functional properties of kinases and their variants are urgently needed. The development of a robust enrichment method with retention of activity, would be a crucial first step for drug discovery and biomarker classification within this class.

KinaSorb™ is a new reagent kit used for the enrichment and isolation of cytosolic (soluble) kinases; it is not suitable for membrane bound (insoluble) kinases. ATP – the common substrate for kinases, is reversibly immobilized to metallic oxide particles, producing a single-use highly efficient enrichment method for kinase & other ATP binding proteins. The standard prep protocol starts with 100 µl of clarified cellular extracts, or approximately 2 mg total cellular extract protein, but the process can be scaled up or down to accommodate different sample volumes and protein concentrations. The kit includes all necessary reagents for immediate use.



*As measured by Universal Protein Tyrosine Kinase assay (Takara Bio Inc, Otsu, Shiga, Japan).

2DE Analysis of KinaSorb™ treated clarified rat brain homogenate. Samples were reduced, alkylated and protein normalized (176 µg total protein per gel). Second dimension was on 8-16% polyacrylamide gels. Circles indicate regions of major protein depletion. ATP binding proteins, and presumably some small carryover proteins are visualized on the right gel. Methods to monitor Kinase drug response are provided in the example below.



In this example, a clarified rat brain homogenate was enriched with KinaSorb™ (box) and then processed through several multi-dimensional separations beads (NRicher™ 6). The bars reflect Kinase kinetic activity* in the various sub-proteome fractions as a percentage of activity in the load applied to the surfaces. The numbers designate the different surface architectures. FT: Flow through, E: elution, L: low Imatinib inhibitor concentration, H: high Imatinib inhibitor concentration.

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Kit Contains:	KinaSorb™ 10	KinaSorb™ 50
Activated KinaSorb™ reagent suspension	0.4 ml	2.0 ml
Cleanascite™ reagent suspension	1 ml	5 ml
KE-BB Binding Buffer	20 ml	100 ml
ATP (dry powder) tube {Store at -20°C}	0.001 gram	0.005 gram
ATP solution buffer	0.25 ml	1.25 ml
KE-EB Elution Buffer	1 ml	5 ml

Storage: Except for ATP (store separately at -20°C), remaining reagents at 4°C

Product	Size	Item No.
KinaSorb™	10 Preps	KE785-10
KinaSorb™	50 Preps	KE785-50
Note: Please contact sales@biotechsupportgroup.com for prices in bulk amount.		

PROTOCOL

Sample Preparation: Avoid use of Phosphate buffers in the cell or tissue extracts. The ideal pH for samples should be around 7.

Each prep processes approximately 1- 2 mg total soluble protein, based on 100 µl of tissue homogenates with a soluble protein content in the 10 – 15 mg/ml range. Larger volumes of lower protein content can also be used, but sample preparation volumes should be proportioned accordingly. Delipidation is recommended for most samples as phospho-lipids can complete with binding. We recommend the use of **Cleanascite**™ supplied as part of the kit, as a useful general clarification and delipidation reagent. Lipid biomass can vary greatly, so the ratios shown are only intended to provide general guidance in use. Resuspend **Cleanascite**™ by gentle shaking.

1. Add 1 volume of **Cleanascite**™ to 5 volumes of the sample. Mix the sample for 10 minutes.
2. Centrifuge sample at 16,000 G's (maximum microcentrifuge).

The supernatant should be clarified and free from colloidal biomass. If still visually colloidal, repeat **Cleanascite**™ step #1. Pipette off "delipidated sample" and use as load to **ATP-KinaSorb**™.

Note – Before performing the next ATP immobilization step, note that for best results, the immobilized **ATP-KinaSorb**™ suspension should be used within 24 hours for best results. So only use as much as is necessary for immediate use.

Surface ATP Immobilization: Shake the product suspension well, then pipette 40 µl of **Activated KinaSorb**™ suspension (as supplied in the kit) into a microcentrifuge tube. Micro-centrifuge the **KinaSorb**™ suspension on medium (5000-8000rpm) setting for 2 minutes and pipette off the supernatant. Add the ATP solution buffer to the ATP dry powder and mix for 10 minutes. To each prep, add 25 µl of the ATP solution to the **Activated KinaSorb**™ pellet. Resuspend well and mix for 1 hour. Any unused portion of the ATP solution should be stored at -20°C. The immobilized **ATP-KinaSorb**™ suspension is used for the remainder of the protocol.

Kinase Enrichment

1) Micro-centrifuge the immobilized **ATP-KinaSorb**™ suspension on medium setting for 2 minutes. Pipette off supernatant.
2) Add 1 ml of **KE-BB** Binding Buffer to pellet.
3) Mix well by pipetting up/down, or light vortexing, or mixing until pellet is homogeneously resuspended.
4) Micro-centrifuge on medium setting for 2 minutes. Discard supernatant.
5) Add 200 µl of **KE-BB** Binding Buffer to the pellet, and 100 µl of the "delipidated sample" prepared from above. If a larger volume of "delipidated sample" is used, proportion **KE-BB** accordingly. Mix well by stirring, pipetting up/down or light vortexing or mixing until pellet is **homogeneously resuspended**. Shake the sample for 10 minutes.
6) Micro-centrifuge on medium setting for 2 minutes. Carefully pipette off the kinase-depleted supernatant {the Flow-Through fraction} as much as possible without disrupting the pellet. **The bead is now enriched with kinases. For on-bead digestion for LC-MS work see on-bead digestion protocol, otherwise proceed to the next step.**
7) Elute with 100 µl **KE-EB** Elution Buffer. Mix well by stirring, pipetting up/down or light vortexing or mixing until pellet is **homogeneously resuspended**. Shake the sample for 30 minutes.
8) Micro-centrifuge on maximum setting for 3 minutes. Collect supernatant, the enriched kinase fraction.

For optimal results, the volumes may need to be adjusted up or down to account for differences in specific activity and other sample matrix factors. The elution buffer is pH 9 so activity measurements must compensate for either higher pH, dilutions to neutrality, or buffer exchange.

On-Bead Digestion Protocol for LC-MS Analysis

- After the final wash steps from step 6, add 100 µls of 5 mM DTT solution to the beads for complete immersion, mix and incubate at 60°C for ½ hour.
- After cooling, add 100 µls of 25 mM iodoacetamide to the DTT/bead suspension, mix and incubate in the dark for 1 hour.
- Centrifuge at 5000xg (medium setting, not max) for 2 mins, and discard supernatant. Transfer the filter slurry of beads, DTT and iodoacetamide to a clean Eppendorf tube.
- On-bead digestion is done by adding 100 µls of a 0.025 ug/uL solution of MS-grade. Trypsin to the beads. Digest overnight at 37°C.
- Centrifuge at 5000xg (medium setting, not max) for 2 mins, and retain peptide filtrate.
- To further extract remaining peptides, add 100 µls of 10% solution of formic acid to the beads.
- Incubate for 15 minutes at 37°C, centrifuge at 15000xg (maximum setting) for 3 mins, and add this volume to the first volume.
- Reduce to a final volume of 100 µls using a SpeedVac and store at -80 °C until LC-MS/MS.

CONTACT US

We welcome your questions and comments regarding our products.

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