USER GUIDE

novex® by life technologies™

ELISA Technical Guide

General information and guidelines for using Novex® ELISA Kits

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Contents

Product Information

About ELISA Kits

Introduction

The Novex® ELISA kits from Life Technologies are based on solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA). The ELISA Kit is used for the quantitative determination of secreted proteins such as cytokines and chemokines, as well as intracellular signaling proteins.

ELISAs are adaptable to high-throughput screening because results are rapid, consistent and relatively easy to analyze. The best results are obtained with the sandwich format, utilizing highly purified, prematched capture and detector antibodies. The resulting signal provides data which is consistent and highly specific.

Shipping and storage

The ELISA Kits are shipped at 2 to 8ºC. Upon receipt, store the kits at 2 to 8ºC. Some components may need storage at –20°C.

Contents

The ELISA Kits include all the reagents needed to perform a sandwich ELISA. This includes the capture antibody-coated 96-well plates, calibrated standard, Wash and Diluent buffers, detection antibody, secondary detection reagents, stabilized chromogen, stop solution, and plate covers.

For detailed kit contents, refer to the product insert supplied with the product.

For research use only. Not intended for any animal or human therapeutic or diagnostic use.

Types of ELISA kits (precoated plates)

- For extracellular proteins, ELISA kits are available for human, mouse, rat, monkey, bovine, and swine samples. These kits are available in the following formats:
  - Colorimetric ELISA kits that offer picogram detection range
  - Ultrasensitive kits for low picogram detection range
  - EASIA™ kits for use with clinical serum or plasma samples for picogram detection range
  - Chemiluminescence ELISA kits that offer low picogram detection range and an increased dynamic range over colorimetric ELISA kits.
- For intracellular proteins for signaling studies, phosphoELISA™ kits are available for measurement of total and phosphorylation, modification, or cleavage site-specific proteins.

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System overview

Novex® ELISA kits are based on the solid phase sandwich ELISA technique. For this method, an antibody against the specific antigen is coated onto the wells of the microtiter strips provided in the ELISA kits.

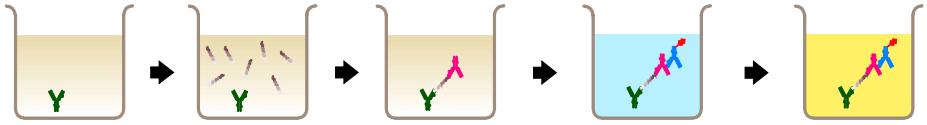
During the first incubation, standards of known content, controls, and unknown samples are added to the coated wells to allow the antigen from the samples bind to the immobilized (capture) antibody.

After washing, a detection antibody (antigen-specific rabbit antibody or biotinylated antibody) is added that binds to the immobilized antigen captured during the first incubation. In some cases, the wash step is omitted and the detection antibody is added at the same time as the samples.

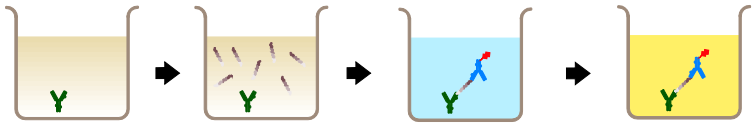
After removal of excess detection antibody, a horseradish peroxidase-labeled Anti-Rabbit IgG (Anti-Rabbit IgG HRP) or Streptavidin-HRP (enzyme) is added. This binds to the detection antibody to complete the four-member sandwich.

After a second incubation and washing to remove all the unbound enzyme, a stabilized substrate solution is added, which is acted upon by the bound enzyme to produce color. The intensity of this colored product is directly proportional to the concentration of antigen present in the original specimen.

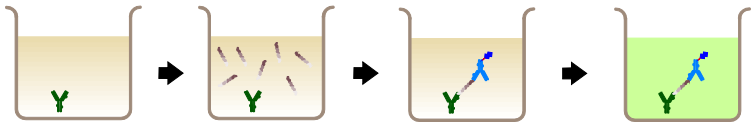
The incubation times takes about 4 hours total and requires only 30 minutes hands-on time.



For the EASIA™ kits, the technique is similar, but the detector antibody is directly conjugated to HRP.



For chemiluminescent sandwich ELISA kits, the protocol is similar with respect to the capture antibody. The difference is that the detector antibody is conjugated to alkaline phosphatase (AP). After rinsing, a substrate, CSPD-Emerald II, is added and a chemiluminescence readout is captured at the end. The incubation time is 3.5 hours.



Assay optimization

There is no need for optimization of precoated ELISA assays as each and every step of the assay is optimized. This is one of the major benefits of using precoated plates. However, it is very important to follow all steps of the protocol, especially the recommended washing steps and incubation times to obtain the best results.

Methods

Before You Begin

The ELISA kits contain materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state, and local regulations for disposal.

Materials required but not provided

- Microtiter plate reader capable of measurement at or near 450 nm (for colorimetric kits)
- Microtiter plate reader (luminometer) capable of chemiluminescence measurement (for chemiluminescent kits)
- Cell Extraction Buffer (Cat. no. FNN0011) for preparation of cell lysates only
- Calibrated adjustable precision pipettes, preferably with disposable plastic tips. (A manifold multi-channel pipette is desirable for large assays.)
- Distilled or deionized water
- Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.)
- Data analysis and graphing software
- Glass or plastic tubes for diluting and aliquoting
- Standard 37°C incubator

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Procedural guidelines

- Refrigerate kit when not in use. Warm all reagents to room temperature before use.
- **Allow the microtiter plates to equilibrate to room temperature before opening the foil bags.** Once the desired number of strips are removed, immediately reseal the bag and store the plate at 2 to 8°C to maintain plate integrity.
- Collect samples in pyrogen/endotoxin-free tubes.
- Samples should be frozen if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.
- When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particulate matter are present, centrifuge or filter sample prior to analysis.
- Run all standards, controls, and samples in duplicate.
- When pipetting reagents, maintain a consistent order of addition from well-to-well to ensure equal incubation times for all wells.
- Cover or cap all reagents when not in use.
- Do not mix or interchange different reagent lots from various kit lots.
- Do not use reagents after the kit expiration date.
- For best results, read absorbances within 30 minutes of assay completion. Plates can be read up to 2 hours after assay completion when kept in the dark.
- The controls provided should be run with every assay. If control values fall outside pre-established ranges, the accuracy of the assay is suspect.
- Drain residual wash liquid from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. ***Never*** insert absorbent paper directly into the wells.
- Because Stabilized Chromogen is light sensitive, avoid prolonged exposure to light.
- Avoid contact between chromogen and metal (e.g., aluminum foil), or color may develop.
- Protect CSPD-Emerald Substrate from prolonged exposure to light.

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Plate washing directions

Perform all washing with the *Wash Buffer Concentrate (25X)* supplied with the kit. Dilute the 25X stock (see page 9) before use as described below. **Incomplete washing adversely affects the results**.

- **Manual Washing**
  Completely aspirate the liquid from all wells by gently lowering an aspiration tip into the bottom of each well. Take care not to scratch the inside of the well. After aspiration, fill the wells with at least 0.4 mL diluted *Wash Buffer*. Allow the buffer to stand for 15 to 30 seconds, and then aspirate the liquid. Repeat as directed under Assay Procedure. After the washing procedure, invert the plate and tap dry on an absorbent tissue.
- **Washing with a Squirt Bottle**
  Place the diluted *Wash Buffer* into a squirt bottle. Flood the plate with the diluted *Wash Buffer*, completely filling all wells. After the washing procedure, invert the plate and tap dry on an absorbent tissue.
- **Automated Plate Washer**
  If using an automated washer, follow the manufacturer's instructions for plate washing.

Procedure limitations

- Do not extrapolate the standard curve beyond the top point of the standard; the dose-response is non-linear in this region and accuracy is difficult to obtain. Dilute samples with concentrations exceeding the linear portion of the standard curve with the Standard Diluent Buffer; reanalyze these and multiply results by the appropriate dilution factor.
- The influence of various drugs and the use of biological fluids in place of tissue culture media have not been thoroughly investigated. The rate of degradation of native protein in various matrices has not been investigated. The immunoassay literature contains frequent references to aberrant signals seen with some sera, attributed to heterophilic antibodies. Though such samples have not been seen to date, the possibility of this occurrence cannot be excluded.

Prepare Reagents and Samples

Dilute wash buffer

1. Allow the Wash Buffer Concentrate (25X) to reach room temperature and mix gently to ensure that any precipitated salts have redissolved before diluting.
2. Dilute 1 volume of the Wash Buffer Concentrate (25X) with 24 volumes of deionized water (e.g., 50 mL may be diluted up to 1.25 liters, 100 mL may be diluted up to 2.5 liters). Label as Working Wash Buffer.
3. Store both the concentrate and the Working Wash Buffer in the refrigerator. Use the diluted buffer within 14 days.

Dilute the standards

Note: Use glass or plastic tubes for standard dilutions.

Reconstitute standard with Standard Diluent Buffer. Refer to the standard vial label for instructions.
Make serial dilutions of the standard as described in the product insert supplied with the product.

Prepare secondary antibody

Note: The anti-rabbit HRP or Streptavidin-HRP (100X) is in 50% glycerol. This solution is viscous. To ensure accurate dilution,

1. Allow the reagent to reach room temperature. Gently mix.
2. Pipette 10 µL (100X) solution. Wipe the pipette tip with clean absorbent paper to remove any excess solution and transfer the solution to a tube containing 990 µL Diluent for each 8-well strip used in the assay, and mix well. Label appropriately.
3. Return the unused reagent to the refrigerator.
4. Use the diluted antibody within 15 minutes.

Prepare samples

See **Sample Preparation and Handling** in **Appendix** for some methods to prepare samples.

Perform the ELISA

ELISA procedure

Review Procedural Notes section before starting.

1. Allow all reagents to reach room temperature before use. Gently mix all liquid reagents prior to use.

   Important: Run a standard curve with each assay.

2. Perform the ELISA procedure as described in the product insert supplied with the product.

Read the plate and generate the standard curve

1. For colorimetric kits, read the absorbance of each well at 450 nm. Read the plate within the recommended time after adding the Stop Solution.
2. For chemiluminescent kits, read the luminescence 30 minutes after the addition of the substrate with a 1000 msec integration time.
3. Use curve-fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit.
4. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

   Note: Dilute samples producing signals greater than that of the highest standard in Standard Diluent Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.

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Troubleshooting

Observation	Cause	Solution
Elevated background	Insufficient washing and/or draining of wells after washing. Solution containing Anti-Rabbit IgG HRP or Streptavidin-HRP can elevate the background if residual is left in the well.	Wash according to the protocol. Verify the function of automated plate washer. At the end of each washing step, invert plate on absorbent tissue on countertop and allow to completely drain, tapping forcefully if necessary to remove residual fluid.
	Contamination of substrate solution with metal ions or oxidizing reagents.	Use distilled/deionized water for dilution of wash buffer and use plastic equipment. **Do not cover** plate with foil.
	Chromogen exposed to light prior to use, resulting in a blue color.	Keep chromogen in vial until ready to dispense into plate and then pour into a reservoir to prevent contamination of the vial with equipment. **Do not cover** plate with foil.
	Incubation time is too long or incubation temperature is too high.	Reduce incubation time and/or temperature.
	Contamination of pipette, dispensing reservoir or substrate solution with Anti-Rabbit IgG HRP or Streptavidin-HRP.	Do not use chromogen that appears blue prior to dispensing onto the plate. Obtain new vial of chromogen.
	Improperly set up blanks.	Follow protocol when designating blank wells. Blank wells contain only chromogen and stop solution and subtract results from all other wells.
	Incorrect dilution of standard stock solution or standards diluted in serum, culture supernatant or other.	Follow the protocol instructions regarding the standard dilution. Dilute standards **only** in Standard Diluent Buffer provided in the kit.
Elevated optical density reading for standard or sample	Incorrect dilution of the Anti-Rabbit IgG HRP or Streptavidin-HRP Working solution.	Warm solution of Anti-Rabbit IgG HRP or Streptavidin-HRP (100X) to room temperature, draw up slowly and wipe tip with laboratory wipe to remove excess. Dilute **only** in HRP diluent provided.
	Incubation times extended.	Follow incubation times outlined in protocol.
	Incubations performed at 37°C.	Perform incubations at room temperature (~25±2°C) when instructed in the protocol.

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Troubleshooting, Continued

Observation	Cause	Solution
Weak or no color development	Reagents not at room temperature (~25±2°C) at start of assay.	Allow ALL reagents to warm to room temperature prior to commencing assay.
	Incorrect storage of components, e.g., not stored at 2 to 8°C.	Store all components exactly as directed in the protocol and on labels.
	Anti-Rabbit IgG HRP or Streptavidin-HRP Working solution made up longer than 15 minutes before use in assay.	Use the diluted Anti-Rabbit IgG HRP or Streptavidin-HRP within 15 minutes of dilution.
	Expired reagents used.	Check expiration dates upon receipt of kit and use the kit prior to expiration.
	Plate read at incorrect wavelength.	The correct wavelength to read ELISA using the TMB substrate is 450 nm.
	TMB solution lost activity.	Ensure the TMB solution is clear before it is dispensed into the microtiter plate wells. An intense aqua blue color indicates that the product is contaminated. Please contact Technical Support if this problem is noted. To avoid contamination, we recommend that the quantity required for an assay be dispensed into a disposable trough for pipetting. Discard any TMB solution left in the trough. Avoid contact of the TMB solution with items containing metal ions.
	Attempt to measure analyte in a matrix for which the ELISA assay is not optimized.	Contact Technical Support when using alternative sample types.
	Wells have been scratched with pipette tip or washing tips.	Use caution when dispensing and aspirating into and out of microwells.
	Incorrect chromogen or stop solution used.	Use only the chromogen and stop solution supplied with the kit.
	Standard Diluent Buffer added to all wells rather than the designated wells.	Follow the protocol and only add standard diluent to the designated wells and to the samples where it is required or to samples producing signals greater than that of the highest standard.
	Used buffer containing azide which is not compatible with HRP.	Avoid the use of azide in the assay.

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Troubleshooting, Continued

Observation	Cause	Solution
Poor standard curve	Improper preparation of standard stock solution.	Dilute lyophilized standard as directed by the vial label only with the Standard Diluent Buffer or in a diluent that most closely matches the matrix of your sample.
	Reagents (lyophilized standard, standard diluent buffer, etc.) from different kits, either different analyte or different lot number, were substituted.	Never substitute any components from another kit.
	Errors in pipetting the standard or subsequent steps.	Always dispense into wells quickly and in the same order. Do not touch the pipette tip on the individual microwells when dispensing. Use calibrated pipettes and the appropriate tips for that device.
Poor precision	Errors in pipetting the standards, samples or subsequent steps.	Always dispense into wells quickly and in the same order. Do not touch the pipette tip on the individual microwells when dispensing. Use calibrated pipettes and the appropriate tips for that device. Check for any leaks in the pipette tip.
	Repetitive use of tips for several samples or different reagents.	Use fresh tips for each sample or reagent transfer.
	Wells have been scratched with the pipette tip or washing tips.	Use caution when dispensing and aspirating into and out of microwells.
	Liquid transferred from well to well during incubations.	Adjust orbital shaker or check for correct rotator rpm. Peel adhesive plate cover off carefully.
	Incorrect volumes of materials dispensed into microwells.	Follow protocol for reagent dispensing volumes. Check calibration of pipettes.
	Standard diluted with the serum, culture medium or other buffer.	Dilute the standard with the standard diluent buffer provided in the kit.
	Particulates or precipitates found in the samples.	Remove any particulates/precipitates by centrifugation prior to dispensing into the assay.
	Dirty microwells-visible debris within or on bottom of microwells.	Inspect microwells and invert plate to remove debris. Wipe bottom of plate with a absorbent tissue after each wash step. Never insert tissue into microwells.
	"Edge effect"-due to uneven temperature from the outer edge wells to the wells in the center of the plate.	Seal the plate completely with cover during incubations and place plate in center of incubator when 37°C incubation is indicated.

Appendix A

Sample Preparation and Handling

Sample preparation methods

Some sample preparation methods for routine samples are described below. Additional sample preparation methods such as rat liver, rat lung, and brain tissue are described in the ELISA Handbook available at **www.lifetechnologies.com**.

Prepare serum

1. Collect whole blood in a covered test tube.
2. After collecting whole blood, allow the blood to clot by leaving it undisturbed at room temperature (usually 15 –30 minutes.
3. Remove the clot by centrifuging at 1000 –2000 × *g* for 10 minutes in a refrigerated centrifuge.
4. The resulting supernatant is serum. Immediately transfer the serum into a clean polypropylene tube using a Pasteur pipette.
5. Maintain the samples at 2 to 8°C while handling. If the serum is not analyzed immediately, aliquot the serum into 0.5 mL aliquots. Store and transport the serum aliquots at –20°C or lower.

   Note: Avoid freeze-thaw cycles because this is detrimental to many serum components.

Prepare plasma

1. Collect whole blood into commercially available anticoagulant-treated tubes e.g., EDTA-treated or citrate-treated.

   Note: Heparinized tubes are indicated for some applications; however, heparin may be contaminated with endotoxin, which can stimulate white blood cells to release cytokines.
2. Remove cells from plasma by centrifugation for 10 minutes at 1000 –2000 × *g* using a refrigerated centrifuge.
3. Centrifuge for 15 minutes at 2000 × *g* to deplete platelets in the plasma sample.
4. The resulting supernatant is designated plasma. Immediately transfer the plasma into a clean polypropylene tube using a Pasteur pipette.
5. Maintain the samples at 2 to 8°C while handling. If the plasma is not analyzed immediately, aliquot the plasma into 0.5 mL aliquots. Store and transport the plasma aliquots at –20°C or lower.

   Note: Avoid freeze-thaw cycles.

Prepare tissue culture medium

1. Following the end of the desired cell culture time, pipette medium into a microcentrifuge tube and immediately place the tube on ice.
2. Centrifuge the sample at 1400 rpm for 1 minute.
3. Transfer supernatant and aliquot into a clean microcentrifuge tube.
4. Store at –80°C until ready for use in ELISA.

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Nuclear extraction method

This protocol has been successfully applied to several cell lines of human origin.

1. Prepare the recommended 1X Hypotonic Buffer:
   - 20 mM Tris-HCl, pH 7.4
   - 10 mM NaCl
   - 3 mM $MgCl_2$
2. Collect cells (5×10^6) in PBS by centrifugation (non-adherent) or scraping from culture flasks (adherent).
3. Wash cells twice with cold PBS.
4. Remove and discard the supernatant and transfer the cells into a prechilled microcentrifuge tube.
5. Gently resuspend cells in 500 µL 1X Hypotonic Buffer by pipetting up and down several times. Incubate on ice for 15 minutes.
6. Add 25 µL detergent (10% NP40) and vortex for 10 seconds at highest setting.
7. Centrifuge the lysate for 10 minutes at 3000 rpm at 4°C.
8. The supernatant contains the cytoplasmic fraction (save the supernatant). The pellet is the nuclear fraction.
9. Resuspend nuclear pellet in 50 µL Cell Extraction Buffer with protease inhibitors (page 16) for 30 minutes on ice with vortexing at 10 minute intervals.
10. Centrifuge for 30 minutes at 14,000 × *g* at 4°C. Transfer supernatant (nuclear fraction) to a clean microcentrifuge tube.
11. Aliquot and store the nuclear extracts at –80°C until use.

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Cell extraction protocol

1. Prepare the required amount of Cell Extraction Buffer (Cat. no. FNN0011) with the following protease inhibitors just prior to use:
   - 1 mM PMSF (stock is 0.3 M in DMSO)
   - Protease inhibitor cocktail (e.g. Sigma Cat. no. P-2714, reconstitute according to manufacturer's guideline). Add 500 µL per 5 mL Cell Extraction Buffer.

   Note: The stability of Cell Extraction Buffer with protease inhibitors is 24 hours at 4°C. PMSF is very unstable and must be added prior to use, even if added previously.
2. Estimate cell density for suspension cells by counting in a hemacytometer.

   Estimate cell density for adherent cells by visual inspection under a microscope. Use cells at 70–80% confluence levels.
3. Stimulate cells as desired.
4. Transfer the cells into clean 15 mL conical tubes:
5. Collect the cells by centrifugation at 300 × *g* for 7 minutes. Aspirate the medium.
6. Resuspend the pellet in ice-cold PBS.
7. Collect the cells by centrifugation at 300 × *g* for 7 minutes at 4°C. Aspirate the PBS.
8. Lyse the cells by pipetting Cell Extraction Buffer with protease inhibitors from step 1 into each tube. Use 1 mL Complete Cell Extraction Buffer per 10^8 cells.
9. Transfer the lysates to clean microcentrifuge tubes.
10. Vortex the mixture, then incubate the mixture on ice for 30 minutes, with occasional vortexing.
11. Clarify the lysates by centrifugation at 13,000 × *g* at 4°C for 10 minutes.
12. Transfer the clarified cell extracts to clean microcentrifuge tubes.
13. Store the clarified cell extracts at –80°C until use. Avoid repeated freeze-thaw cycles.

Determine protein concentration using a suitable method, such as the Quant-iT™ kit (Cat. no. Q33210). Cell extracts prepared by this method routinely have a protein concentration between 1 and 10 µg/mL.

All cell extracts require dilution by a factor of at least 1:10 in Standard Diluent Buffer before analysis.

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Tissue homogenate protocol

1. Prepare the required amount of Cell Extraction Buffer (Cat. no. FNN0011) with the following protease inhibitors just prior to use:
   - 1 mM PMSF (stock is 0.3 M in DMSO)
   - Protease inhibitor cocktail (e.g. Sigma Cat. no. P-2714, reconstitute according to manufacturer's guideline). Add 500 µL per 5 mL Cell Extraction Buffer.

   Note: The stability of Cell Extraction Buffer with protease inhibitors is 24 hours at 4°C. PMSF is very unstable and must be added prior to use, even if added previously.
2. Add ~100 mg of the tissue sample (wet mass) to an Eppendorf tube.
3. Add 8 volumes of Cell Extraction Buffer with protease inhibitors from step 1 to the tube by 50–100 µL increments, and homogenize thoroughly with a Dounce homogenizer or by sonication.
4. Transfer the lysate to a clean microcentrifuge tube.
5. Vortex the mixture, then incubate the mixture on ice for 30 minutes, with occasional vortexing.
6. Clarify the lysate by centrifugation at 13,000 × *g* at 4°C for 10 minutes.
7. Transfer the supernatant to a clean microcentrifuge tube.
8. Determine protein concentration using a suitable method, such as the Quant-iT™ kit (Cat. no. Q33210).
9. Dilute the tissue extract an additional 1:10 to 1:100 with Standard Diluent Buffer before analysis.

Brain tissue homogenate protocol

1. Prepare the required amount of homogenization buffer and protease inhibitors prior to use:
   - 5 M guanidine-HCl diluted in 50 mM Tris, pH 8.0
   - 1X PBS supplemented with 1X protease inhibitor cocktail.
2. Add ~100 mg of the brain tissue sample (wet mass) to an Eppendorf tube.
3. Add 8 volumes of cold 5 M guanidine-HCl in 50 mM Tris to the tube by 50–100 µL increments, and grind thoroughly with a hand-held tissue homogenizer after each addition.
4. (*Optional*) Transfer the homogenate to a 1 mL Dounce homogenizer and homogenize thoroughly.
5. Mix the homogenate on an orbital shaker at room temperature for three to four hours. The sample is stable at this stage, and can undergo multiple freeze-thawed cycles.
6. Dilute the sample ten-fold with cold PBS with 1X protease inhibitor cocktail. Centrifuge at 16,000 × *g* for 20 minutes at 4°C.
7. Carefully transfer the supernatant to a new tube, and place it on ice.
8. Dilute the tissue extract (supernatant) an additional 1:10 to 1:100 with Standard Diluent Buffer prior to application in the ELISA.

Frequently Asked Questions (FAQs)

Question	Answer
What is the difference between Total and phosphoELISA™ kits?	Both assays capture total protein, regardless of phosphorylation state, to the wells of an ELISA plate. This is done by coating the plates with a "pan" antibody that does not distinguish between the phosphorylated and nonphosphorylated forms of a protein and does not block the phosphorylation site to be studied. One assay then quantifies the total amount of protein present in the sample by using a second "pan" antibody for detection of protein regardless of phosphorylation state. A second assay quantifies the amount of that same protein that has been phosphorylated at a specific amino acid. Instead of a second "pan" antibody for detection, this assay uses an antibody that specifically recognizes an epitope that is only present on a protein when it is phosphorylated at a particular amino acid position. This procedure is extremely similar to an immunoprecipitation reaction in which two different antibodies are used. One is used to capture a protein from a solution and one is used to detect the subset of that protein population that is phosphorylated at a certain site.
How does phosphoELISA™ compare to immuno-precipitation and western blotting?	phosphoELISA™ assays have several advantages, including ease of use and increased sensitivity. PhosphoELISAs are typically 2–10X more sensitive than western blots, which is particularly useful for the detection of low-expressing proteins or for small sample sizes. In addition, using the recombinant standards provided in the kit, phosphoELISA™ kits provide quantitative results without having to perform densitometry.
What unit of measurement do I use for phosphoELISA™ data?	The measurement is given in pg/mL of sample, or sometimes ng/mL of protein. The measurement is always given in mass units. This is because standards of known mass are available for use in the standard curve for the total assay. This second measurement is given in "units" which we do not relate to a particular mass of protein. This is done because it is difficult to know precisely the efficiency of a particular phosphorylation reaction, and therefore the ratio of phosphorylated to nonphosphorylated protein in a particular preparation of phosphoprotein standard. Phosphorylation units will be unique to each phosphoELISA™ assay, and are described within the product insert that comes with the assay. For example, a typical unit description would be "1 unit = the amount of AKT[pS473] derived from 100 pg of AKT phosphorylated by MAPKAP2 and PDK1" as is seen for our AKT[pS473] phosphoELISA™ assay. Since there is no guarantee that the AKT in our standard preparation is 100% phosphorylated, we refrain from making the statement that this corresponds to 100 pg of phosphorylated AKT. Instead, we validate a large batch of phosphorylated protein and use this to develop our unit definition and standard curve for our original assay. Subsequent preparations of our protein standards are normalized to the original batch of protein to insure that our unit definitions remain constant from lot to lot.

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Frequently Asked Questions (FAQs), Continued

Question	Answer
How do I normalize phosphoELISA™ results with Total ELISA results?	To evaluate phosphorylation levels, we report comparative levels of phosphorylation of a protein, in units of phosphoprotein per picogram or nanogram of total protein. The total ELISA kit quantifies the mass of protein per sample and the phosphoELISA™ kit quantifies the phosphorylation level of that protein in units. One can then determine if phosphorylation levels (in units/pg, for instance) increase, decrease, or remain constant between various samples.

Ordering Information

Related products

The following products are also available. For more details on these products, visit **www.lifetechnologies.com** or contact **Technical Support**.

Product	Quantity	Catalog no.
Cell Extraction Buffer	100mL	FNN0011
Wash Buffer (25X)	100mL	WB01
Wash Buffer (25X), Bulk Pack	1L bottle	WB02
Stabilized Chromogen	25mL	SB01
Stabilized Chromogen	1L bottle	SB02
Stop Solution	25mL	SS03
Stop Solution	1L bottle	SS04

Appendix B

Safety

Biological safety

Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

In the U.S.:

- U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories found at: **www.cdc.gov/biosafety**
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.

Additional information about biohazard guidelines is available at: www.cdc.gov

In the EU:

Check local guidelines and legislation on biohazard and biosafety precaution and refer to the best practices published in the World Health Organization (WHO) Laboratory Biosafety Manual, third edition, found at: **www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/**

Technical Support

Obtaining support

For the latest services and support information for all locations, go to **www.lifetechnologies.com**.

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (**techsupport@lifetech.com**)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Safety Data Sheets (SDS)

Safety Data Sheets (SDSs) are available at **www.lifetechnologies.com/support**.

Certificate of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to **www.lifetechnologies.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the box.

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Explanation of Symbols

Symbol	Description	Symbol	Description
REF	Catalog Number	LOT	Lot/Batch code
RUO	Research Use Only		Temperature limitation
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