AKT (Total) ELISA Kit

Catalog Number KHO0101 (96 tests)

Pub. No. MAN0014948 **Rev.** 3.0 (31)

CAUTION! This kit contains 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.

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Invitrogen™ AKT (Total) ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of AKT in lysates from cells. The assay recognizes both natural and recombinant AKT. Although performance characterization of this ELISA kit was done primarily on human cell lines, cross-reactivity of this kit with mouse and rat cells was observed.

AKT, also known as the protein kinase B-α (PKB-α) or RAC-PKα, was initially identified as one of the downstream targets of PI-3 Kinase (PI3-K). The AKT (Total) ELISA is designed to detect and quantify the levels of AKT protein, independent of its phosphorylation state. This assay is intended to detect AKT from lysates of cells and can be used to normalize the AKT content of the samples when using the AKT [pS473] ELISA Kit (Cat. No. KHO0111).

Contents and storage

Upon receipt, store the kit at 2°C to 8°C.

Contents	Cat. No. KHO0101 (96 tests)
AKT (Total) Standard; lyophilized. Refer to vial label for quantity and reconstitution volume	2 vials
Standard Diluent Buffer; contains 0.1% sodium azide	25 mL
AKT Antibody-Coated Plate, 96-well strip-well plate	1 plate
AKT (Total) Biotin Conjugate; contains 0.1% sodium azide	11 mL
Streptavidin-HRP (100X)	0.125 mL
Streptavidin-HRP Diluent; contains 3.3 mM thymol	25 mL
Wash Buffer Concentrate (25X)	100 mL
Stabilized Chromogen, Tetramethylbenzidine (TMB)	25 mL
Stop Solution	25 mL
Plate Covers, adhesive strips	3

Required materials not supplied

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at or near 450 nm
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions; beakers, flask and cylinders for preparation of reagents
- Cell Extraction Buffer (Cat. No. FNN0011, or see "Prepare Cell Extraction Buffer")

Before you begin

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **thermofisher.com**.
- Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

Prepare 1X Wash Buffer

1. Dilute 16 mL of Wash Buffer Concentrate (25X) with 384 mL of deionized or distilled water. Label as 1X Wash Buffer.
2. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 14 days.

Prepare Cell Extraction Buffer

Note: See the *ELISA Technical Guide* for detailed information on preparing Cell Extraction Buffer.

1. Prepare Cell Extraction Buffer.

   Cell Extraction Buffer consists of 10 mM Tris (pH 7.4), 100 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM NaF, 20 mM $Na_4P_2O_7$, 2 mM Na_3VO_4, 1% Triton™ X-100, 10% glycerol, 0.1% SDS, and 0.5% deoxycholate.
2. Immediately before use, add PMSF (0.3 M stock in DMSO) to 1 mM and 50 µL protease inhibitor cocktail (e.g., Sigma Cat. No. P-2714) for each 1 mL of Cell Extraction Buffer.

Prepare cell lysate

1. Collect cells by centrifugation (non-adherent cells) or scraping from culture flasks (adherent cells), then wash cells twice with cold PBS.
2. Remove and discard the supernatant and collect the cell pellet. The pellet can be stored at −80°C and lysed at a later date if desired.
3. Lyse the cell pellet in Cell Extraction Buffer for 30 minutes, on ice. Vortex at 10-minute intervals.

   Note: The volume of Cell Extraction Buffer used depends on the number of cells in the cell pellet, and expression levels of AKT. Researchers must optimize the extraction procedures for their own applications.
4. Transfer the lysate into microcentrifuge tubes and centrifuge at 13,000 rpm for 10 minutes at 4°C.
5. Transfer the supernatant into clean microcentrifuge tubes. Samples can be stored at −80°C (avoid multiple freeze-thaw cycles).

Pre-dilute samples

Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

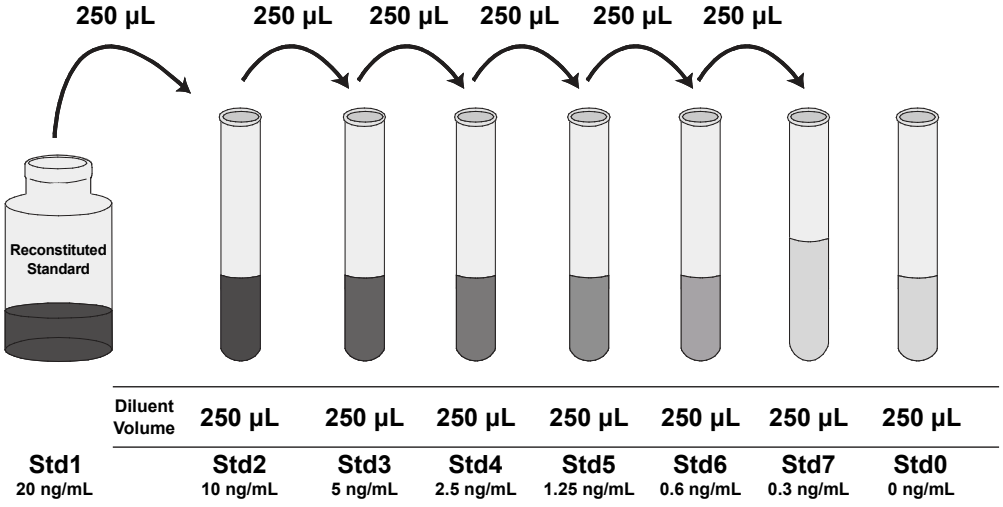
- Dilute samples prepared in Cell Extraction Buffer 1:10 or greater in Standard Diluent Buffer (e.g., 10 µL sample into 90 µL buffer). This dilution is necessary to reduce the matrix effect of the Cell Extraction Buffer. SDS concentration should be less than 0.01% before adding to the plate. While a 1:10 sample dilution has been found to be satisfactory, higher dilutions such as 1:25 or 1:50 may be optimal.
- Perform other sample dilutions with Standard Diluent Buffer.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

Note: This AKT (Total) standard was prepared from purified, full length, recombinant human AKT expressed in S*f*21 cells.

1. Reconstitute AKT (Total) Standard to 20 ng/mL with Standard Diluent Buffer. Refer to the standard vial label for instructions. Swirl or mix gently and allow the contents to sit for 10 minutes to ensure complete reconstitution. Label as 20 ng/mL AKT. **Use the standard within 1 hour of reconstitution.**
2. Add 250 µL Standard Diluent Buffer to each of 7 tubes labeled as follows: 10, 5, 2.5, 1.25, 0.6, 0.3, and 0 ng/mL AKT.
3. Make serial dilutions of the standard as shown in the following dilution diagram. Mix thoroughly between steps.
4. Remaining reconstituted standard should be discarded or frozen in aliquots at –80°C for further use. Standard can be frozen and thawed one time only without loss of immunoreactivity.



Prepare 1X Streptavidin-HRP solution

Note: Prepare 1X Streptavidin-HRP within 15 minutes of usage.

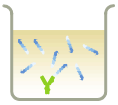
1. For each 8-well strip used in the assay, pipet 10 µL Streptavidin-HRP (100X) solution, and dispense the solution into a tube containing 1 mL of 1X Assay Buffer. Mix thoroughly.
2. Return the unused Streptavidin-HRP (100X) solution to the refrigerator.

Perform ELISA (Total assay time: 4 hours)

IMPORTANT! Perform a standard curve with each assay.

- Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.
- Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 2°C to 8°C for future use.

1 Bind antigen



a. Add 100 µL of standards, controls, or samples (see "Pre-dilute samples" on page 2) to the appropriate wells. Leave the wells for chromogen blanks empty. Tap gently on side of the plate to mix.
b. Cover the plate with a plate cover and incubate for 2 hours at room temperature.
c. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

2 Add Biotin Conjugate

a. Add 100 µL AKT (Total) Biotin Conjugate solution into each well except the chromogen blanks.
b. Cover the plate with plate cover and incubate for 1 hour at room temperature.
c. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

3 Add Streptavidin-HRP

a. Add 100 µL 1X Streptavidin-HRP solution (see page 2) into each well except the chromogen blanks.
b. Cover the plate with a plate cover and incubate for 30 minutes at room temperature.
c. Thoroughly aspirate the solution from the wells and wash wells 4 times with 1X Wash Buffer.

4 Add Stabilized Chromogen

a. Add 100 µL Stabilized Chromogen to each well. The substrate solution begins to turn blue.
b. Incubate for 30 minutes at room temperature in the dark.

Note: TMB should not touch aluminum foil or other metals.

5 Add Stop Solution

Add 100 µL Stop Solution to each well. Tap the side of the plate to mix. The solution in the wells changes from blue to yellow.

Read the plate and generate the standard curve

1. Read the absorbance at 450 nm. Read the plate within 2 hours after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A 4 parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. 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 the upper limit of the standard curve in Standard Diluent Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve example

The following data were obtained for the various standards over the range of 0 to 20 ng/mL AKT.

Standard AKT (Total) (ng/mL)	Optical Density (450 nm)
20	3.38
10	1.84
5	1.26
2.5	0.56
1.25	0.46
0.6	0.31
0.3	0.30
0	0.24

Inter-assay precision

Samples were assayed 48 times in multiple assays to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (ng/mL)	10.9	3.5	1.8
Standard Deviation	1.07	0.3	0.1
% Coefficient of Variation	9.7	9.6	8.7

Intra-assay precision

Samples of known AKT concentration were assayed in replicates of 16 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (ng/mL)	10	3.7	1.4
Standard Deviation	0.7	0.3	0.09
% Coefficient of Variation	6.9	9.9	6.3

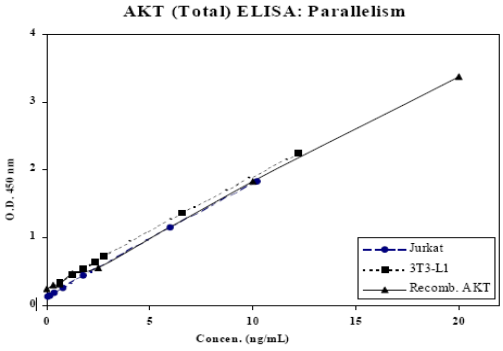
Linearity of dilution

Jurkat cells were grown in tissue culture medium containing 10% fetal bovine serum and lysed with cell extraction buffer. This lysate was diluted in Standard Diluent Buffer over the range of the assay and measured for AKT content. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99 in both cases.

Dilution	Measured (ng/mL)	Expected (ng/mL)	% Expected
Neat	13.9	13.9	100
1/2	6.2	6.9	90
1/4	3.5	3.5	100
1/8	2.1	1.8	116

Parallelism

Natural AKT from Jurkat cells and 3T3-L1 mouse cell extracts was serially diluted in Standard Diluent Buffer. The optical density of each dilution was plotted against the AKT standard curve. The standard accurately reflects AKT content in samples.



Recovery

To evaluate recovery, extraction buffer was diluted 1:10 with Standard Diluent Buffer to bring the SDS concentration <0.01%. Recombinant AKT was spiked into the extract at 3 levels and the % recovery over endogenous levels calculated. On avg., 101% recovery was observed.

Sensitivity

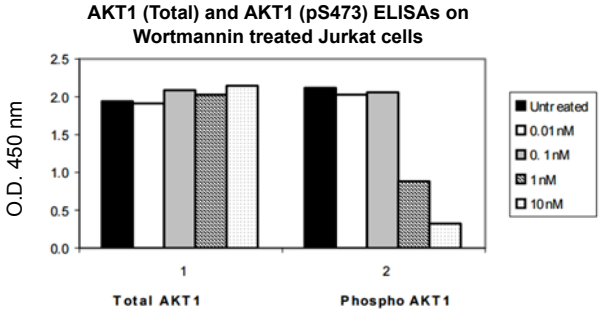
Analytical sensitivity of this assay is <0.1 ng/mL of human AKT. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times. In Jurkat cells cultured in complete medium, this sensitivity corresponded to the AKT protein extractable from 1500 cells. Sensitivity of the ELISA is ~2-fold greater than that of western blot when tested against known quantities of AKT.

ELISA: OD 450 nm	0.146	0.208	0.270	0.465	0.573	0.973	1 .521	2.582
AKT (ng/test)	0	0.03	0.06	0.125	0.25	0.5	1	2

Specificity

The following proteins were tested in the assay at 100 ng/mL and found to have no cross-reactivity: p38 MAPK, p42 ERK1, p42 ERK2, JNK1, human insulin receptor, rat insulin receptor, and human EGFR.

In the figure below, Jurkat cells were treated with wortmannin, a PI3K specific inhibitor, at varying concentrations of 0-500 nM for 3 hours, lysed, and assayed in parallel for both AKT (Total) and AKT [pS473]. The amount of Total AKT remained comparable while the levels of phosphorylation at serine residue 473 decreased with increasing doses of wortmannin. The phosphorylated AKT was analyzed with the Invitrogen AKT [pS473] ELISA Kit (Cat. # KHO0111).



The data indicate that the AKT (Total) ELISA detects both phosphorylated and non-phosphorylated AKT in Jurkat cells, whereas the AKT [pS473] ELISA detects phosphorylated AKT in wortmannin treated cells. This "Total" assay is designed to allow normalization of AKT content among samples to permit interpretation of results from Phosphorylation Site-Specific AKT kits available from Invitrogen.

Limited product warranty

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Product label explanation of symbols and warnings

REF	Catalog Number	LOT	Batch code		Temperature limitation		Use by		Manufacturer		Consult instructions for use		Caution, consult accompanying documents

Manufacturer's address: Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria

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