AKT1 (Total) ELISA Kit

Catalog Number KHO0531 (96 tests)

Pub. No. MAN0014547 **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™ AKT1 (Total) ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of AKT1 (total) in lysates of cells and quantify the level of AKT1 (total) protein, independent of its phosphorylation state and can be used to normalize the AKT1 (total) content of the sampleswhen examining quantities of phosphorylated sites on AKT1 (total) using other kits. The assay will recognize both natural and recombinant AKT1 (total).

Contents and storage

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

Contents	Cat. No. KHO0531 (96 tests)
AKT1 (Total) Standard. Lyophilized; contains 0.1% sodium azide.	2 vials
Standard Diluent Buffer; contains 0.1% sodium azide, red dye [1]	25 mL
Antibody Coated Wells, 96-well plate	1 plate
AKT1 (Total) Detection Antibody. Contains 0.1% sodium azide, blue dye [1].	6 mL
Anti-Rabbit IgG HRP (100X)	0.125 mL
HRP Diluent. Contains 3.3 mM thymol, yellow dye [1]	25 mL
Wash Buffer Concentrate (25X)	100 mL
Stabilized Chromogen, Tetramethylbenzidine (TMB)	25 mL
Stop Solution	25 mL
Adhesive Plate Covers	3

[1] To avoid pipetting mistakes, colored Standard Diluent Buffer, Detection Antibody, and HRP Diluent are provided to monitor the addition of solution to each well. Dyes do not interfere with test results.

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 AKT1 (total). 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.

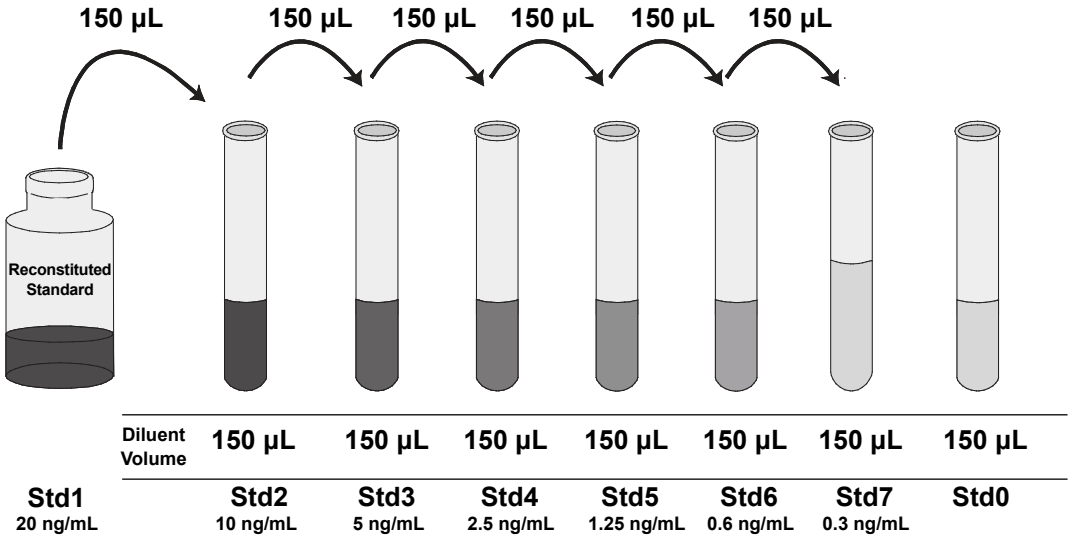
- Perform sample dilutions with Standard Diluent Buffer.
- Dilute samples prepared in cell extraction buffer 5-fold or greater in Standard Diluent Buffer. This dilution is necessary to reduce the matrix effect of the Cell Extraction Buffer. SDS concentration should be less than 0.01% before adding to the plate. While a 1:5 sample dilution has been found to be satisfactory, higher dilutions such as 1:10 or 1:20 may be optimal.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

Note: The AKT1 (Total) Standard is prepared using purified, full length, recombinant, human AKT1 expressed in Sf21 cells.

1. Reconstitute AKT1 (Total) Standard to 20 ng/mL with Standard Dilution 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 AKT1 (total). **Use the standard within 1 hour of reconstitution.**
2. Add 150 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 AKT1 (total).
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. Return Standard Diluent Buffer to the refrigerator.



Prepare 1X Anti-Rabbit IgG HRP solution

Note: Prepare 1X Anti-Rabbit IgG HRP solution within 15 minutes of usage.

1. For each 8-well strip used in the assay, pipet 10 µL Anti-Rabbit IgG HRP (100X) solution, and dispense the solution into a tube containing 1 mL of HRP Diluent. Mix thoroughly.
2. Return the unused Anti-Rabbit IgG 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 and add detector**

a. Add 50 µL of standards, controls, or samples (see "Pre-dilute samples"on page 2) to the appropriate wells. Leave the wells for chromogen blanks empty.
b. Add 50 µL of AKT1 (Total) Detection Antibody solution into each well except the chromogen blanks.
c. Tap the side of the plate to mix. Cover the plate with a plate cover and incubate 3 hours at room temperature.
d. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

2 **Add IgG HRP**

a. Add 100 µL Anti-Rabbit IgG HRP into each well except the chromogen blanks.
b. Cover the plate with plate cover and incubate for 30 minutes at room temperature.
c. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

3 **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.

4 **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 AKT1 (total).

Standard AKT1 (Total) (ng/mL)	Optical Density (450 nm)
20	2.52
10	1.85
5	1.21
2.5	0.77
1.25	0.49
0.6	0.36
0.3	0.27
0	0.15

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)	15.0	7.0	2.6
Standard Deviation	1.4	0.7	0.3
% Coefficient of Variation	9.2	9.4	9.9

Intra-assay precision

Samples of known AKT1 (total) concentration were assayed in replicates of 16 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (ng/mL)	15.0	7.0	2.6
Standard Deviation	1.3	0.6	0.2
% Coefficient of Variation	8.6	9.2	9.1

High-dose hook effect

Samples spiked with AKT1 (total) Standard up to 100 ng/mL give responses higher than that obtained from the highest standard point.

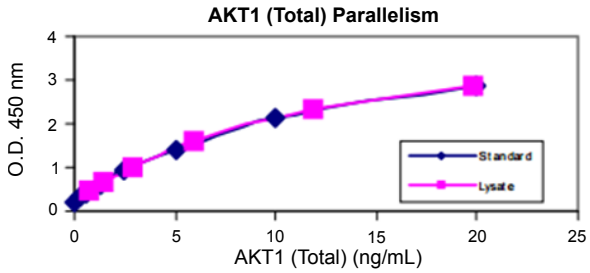
Linearity of dilution

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 AKT1 (total) 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)	%
Neat	3.58	3.58	100
1/2	1.52	1.79	84.9
1/4	0.78	0.90	87.3
1/8	0.43	0.45	95
1/16	0.26	0.22	115.4

Parallelism

Natural AKT1 (total) from Jurkat cell extracts was serially diluted in Standard Diluent Buffer. The optical density of each dilution was plotted against the AKT1 (total) standard curve. The standard accurately reflects AKT1 (total) content in samples.

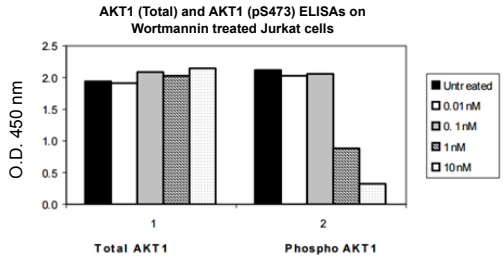


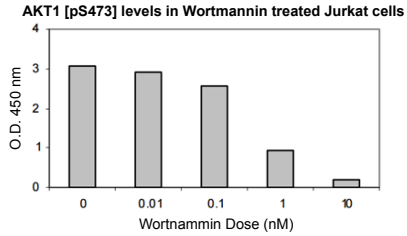
Recovery

To evaluate recovery, Cell Extraction Buffer was diluted 1:5 with Standard Diluent Buffer and spiked with recombinant AKT1 at 3 levels. On average, 70% recovery was observed.

Specificity

The assay specificity for AKT1 (total) was tested with proteins at 100 ng/mL and found to have no cross-reactivity: p38 MAPK, ERK 1/2, FAK, I k B, JNK1, AKT2, AKT3. Jurkat cells were treated with wortmannin, a PI3-K specific inhibitor, at varying concentrations of 0 to 10 nM for 3 hours, lysed, and assayed in parallel for both AKT1 (total) and AKT1 [pS473]. The amount of AKT1 (total) remained comparable while the levels of phosphorylation at serine residue 473 decreased with increasing doses of wortmannin. The phosphorylated AKT1 was analyzed with the AKT1 [pS473] HS ELISA Kit (#KHO0541). The right figure shows the normalization of the AKT1 [pS473] HS ELISA Kit (#KHO0541) by the AKT1 (Total) ELISA Kit (#KHO0531) on wortmannin treated Jurkat cells.





Sensitivity

Analytical sensitivity of this assay is <0.2 ng/mL AKT1 (total). This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times, and calculating the corresponding concentration. In Jurkat cells cultured in complete medium, this sensitivity corresponded to the AKT1 protein extractable from 1,500 cells.

The sensitivity of this ELISA is approximately 2 times greater than that of Western blotting when tested against known quantities of AKT1 (total).

ELISA: OD 450 nm	3.076	2.577	1.882	1.234	0.769	0.501	0.363	0.207
AKT1 (ng/test)	2.0	1.0	0.5	0.25	0.125	0.06	0.03	0

Limited product warranty

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