Datasheet for ABIN415150

Ki-67 ELISA Kit

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Overview

Quantity:	96 tests
Target:	Ki-67 (MKI67)
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	0.78 ng/mL - 50 ng/mL
Minimum Detection Limit:	0.78 ng/mL
Application:	ELISA

Product Details

Purpose:	The kit is a sandwich enzyme immunoassay for in vitro quantitative measurement of Ki67P in human tissue homogenates, cell lysates, cell culture supernates. We offer **validation data** (WB) **for the kit components**. So you can be sure to order a reliable ELISA kit product composed of high quality reagents.
Sample Type:	Cell Culture Supernatant, Cell Lysate, Tissue Homogenate
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This assay has high sensitivity and excellent specificity for detection of Ki-67 Protein (Ki67P). No significant cross-reactivity or interference between Ki-67 Protein (Ki67P) and analogues was observed.
Cross-Reactivity (Details):	No significant cross-reactivity or interference between Ki-67 Protein (Ki67P) and analogues was

Product Details

	observed.
Sensitivity:	0.32 ng/mL
Components:	• Pre-coated, ready to use 96-well strip plate, flat buttom • Plate sealer for 96 wells • Reference Standard • Standard Diluent • Detection Reagent A • Detection Reagent B • Assay Diluent A • Assay Diluent B • Reagent Diluent (if Detection Reagent is lyophilized) • TMB Substrate • Stop Solution • Wash Buffer (30 x concentrate) • Instruction manual

Target Details

Target:	Ki-67 (MKI67)
Alternative Name:	Ki67P (MKI67 Products)
UniProt:	P46013
Pathways:	Glycosaminoglycan Metabolic Process

Application Details

Application Notes:	• Limited by the current condition and scientific technology, we cannot completely conduct the comprehensive identification and analysis on the raw material provided by suppliers. So there might be some qualitative and technical risks to use the kit. • The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments. Please make sure that sufficient samples are available. • Kits from different batches may be a little different in detection range, sensitivity and color developing time. • Do not mix or substitute reagents from one kit lot to another. Use only the reagents supplied by manufacturer. • Protect all reagents from strong light during storage and incubation. All the bottle caps of reagents should be covered tightly to prevent the evaporation and contamination of microorganism. • There may be some foggy substance in the wells when the plate is opened at the first time. It

will not have any effect on the final assay results. Do not remove microtiter plate from the storage bag until needed.

- Wrong operations during the reagents preparation and loading, as well as incorrect parameter setting for the plate reader may lead to incorrect results. A microplate plate reader with a bandwidth of 10nm or less and an optical density range of 0-3 O.D. or greater at 450 ± 10nm wavelength is acceptable for use in absorbance measurement. Please read the instruction carefully and adjust the instrument prior to the experiment.
- Even the same operator might get different results in two separate experiments. In order to get better reproducible results, the operation of every step in the assay should be controlled. Furthermore, a preliminary experiment before assay for each batch is recommended.
- Each kit has been strictly passed Q.C test. However, results from end users might be inconsistent with our in-house data due to some unexpected transportation conditions or different lab equipments. Intra-assay variance among kits from different batches might arise from above factors, too.
- Kits from different manufacturers for the same item might produce different results, since we have not compared our products with other manufacturers.

Comment:	Information on standard material: The standard might be recombinant protein or natural protein, that will depend on the specific kit. Moreover, the expression system is E.coli or yeast or mammal cell. There is 0.05% proclin 300 in the standard as preservative. Information on reagents: The stop solution used in the kit is sulfuric acid with concentration of 1 mol/L. And the wash solution is TBS. The standard diluent contains 0.02 % sodium azide, assay diluent A and assay diluent B contain 0.01% sodium azide. Some kits can contain is BSA in them. Information on antibodies: The provided antibodies and their host vary in different kits.
Sample Volume:	100 µL
Assay Time:	3 h
Plate:	Pre-coated
Protocol:	The test principle applied in this kit is Sandwich enzyme immunoassay. The microtiter plate provided in this kit has been pre-coated with an antibody specific to Ki-67 Protein (Ki67P). Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated antibody specific to Ki-67 Protein (Ki67P). Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After TMB substrate solution is added, only those wells that contain Ki-67 Protein (Ki67P), biotin-conjugated antibody and

enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450nm ± 10nm. The concentration of Ki-67 Protein (Ki67P) in the samples is then determined by comparing the O.D. of the samples to the standard curve.

Reagent Preparation:

1. Bring all kit components and samples to room temperature (18-25 °C) before use. If the kit will not be used up in one time, please only take out strips and reagents for present experiment, and leave the remaining strips and reagents in required condition.
2. Standard - Reconstitute the Standard with 1.0 mL of Standard Diluent, keep for 10 minutes at room temperature, shake gently (not to foam). The concentration of the standard in the stock solution is 50 ng/mL. Prepare 7 tubes containing 0.5 mL Standard Diluent and produce a double dilution series. Mix each tube thoroughly before the next transfer. Set up 7 points of diluted standard such as 50 ng/mL, 25 ng/mL, 12.5 ng/mL, 6.25 ng/mL, 3.12 ng/mL, 1.56 ng/mL, 0.78 ng/mL, and the last microcentrifuge tube with Standard Diluent is the blank as 0 ng/mL.
3. Detection Reagent A and Detection Reagent B - If lyophilized reconstitute the Detection Reagent A with 150µL of Reagent Diluent, keep for 10 minutes at room temperature, shake gently (not to foam). Briefly spin or centrifuge the stock Detection A and Detection B before use. Dilute them to the working concentration 100-fold with Assay Diluent A and B, respectively.
4. Wash Solution - Dilute 20 mL of Wash Solution concentrate (30x) with 580 mL of deionized or distilled water to prepare 600 mL of Wash Solution (1x).
5. TMB substrate - Aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into the vial again.

Note:

1. Making serial dilution in the wells directly is not permitted.
2. Prepare standards within 15 minutes before assay. Please do not dissolve the reagents at 37 °C directly.
3. Please carefully reconstitute Standards or working Detection Reagent A and B according to the instruction, and avoid foaming and mix gently until the crystals are completely dissolved. To minimize imprecision caused by pipetting, use small volumes and ensure that pipettors are calibrated. It is recommended to suck more than 10µL for one pipetting.
4. The reconstituted Standards, Detection Reagent A and Detection Reagent B can be used only once.
5. If crystals have formed in the Wash Solution concentrate (30x), warm to room temperature and mix gently until the crystals are completely dissolved.
6. Contaminated water or container for reagent preparation will influence the detection result.

Assay Precision:

Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level Ki-67 Protein (Ki67P) were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level Ki-

Application Details

	67 Protein (Ki67P) were tested on 3 different plates, 8 replicates in each plate. CV(%) = SD/meanX100 Intra-Assay: CV<10% Inter-Assay: CV<12%
Restrictions:	For Research Use only

Handling

Precaution of Use:	The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.
Handling Advice:	The stability of kit is determined by the loss rate of activity. The loss rate of this kit is less than 5 % within the expiration date under appropriate storage condition. To minimize extra influence on the performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end.
Storage:	4 °C
Storage Comment:	• For unopened kit: All the reagents should be kept according to the labels on vials. The Standard, Detection Reagent A, Detection Reagent B and the 96-well strip plate should be stored at -20°C upon receipt while the others should be at 4°C. • For opened kit: When the kit is opened, the remaining reagents still need to be stored according to the above storage condition. Besides, please return the unused wells to the foil pouch containing the desiccant pack, and reseal along entire edge of zip-seal. Note: It is highly recommended to use the remaining reagents within 1 month provided this is within the expiration date of the kit. • For ELISA kit, 1 day storage at 37°C can be considered as 2 months at 4°C, which means 3 days at 37°C equaling 6 months at 4°C.
Expiry Date:	6 months

Publications

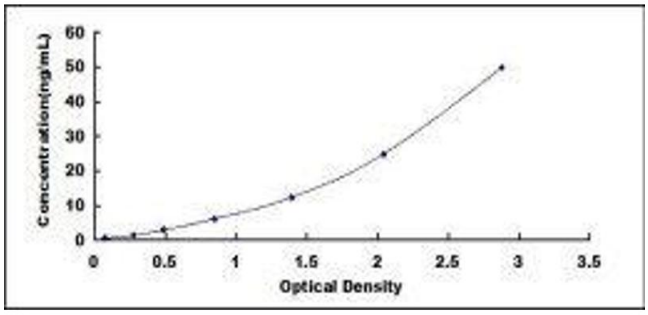
Product cited in:	Tezval, Hansen, Schmelz, Komrakova, Stuermer, Sehmisch: "Effect of Urocortin on strength and microarchitecture of osteopenic rat femur." in: **Journal of bone and mineral metabolism**, Vol. 33, Issue 2, pp. 154-60, (2015) (PubMed). Dai, Zhang, Karatsinides, Keller, Kozloff, Aftab, Schimmoller, Keller: "Cabozantinib inhibits prostate cancer growth and prevents tumor-induced bone lesions." in: **Clinical cancer research**

Publications

: an official journal of the American Association for Cancer Research, Vol. 20, Issue 3, pp. 617-30, (2014) (PubMed).

Kir, Garip, Sahin, Özta?: "Effects of carbamazepine on serum parathormone, 25- hydroxyvitamin D, bone specific alkaline phosphatase, C-telopeptide, and osteocalcin levels in healthy rats." in: **Bosnian journal of basic medical sciences / Udruženje basi?nih mediciniskih znanosti = Association of Basic Medical Sciences**, Vol. 12, Issue 4, pp. 240-4, (2012) (PubMed).

Images



ELISA

Image 1.

Validation report #029832 for ELISA (ELISA)

Successfully validated (ELISA (ELISA))

by Alamo Laboratories

Report Number: 029832

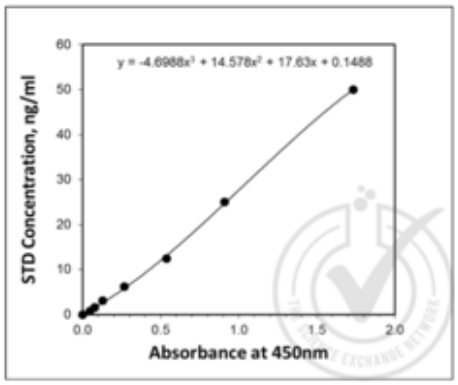
Date: Feb 28 2015

Lot Number:	L141016326
Method validated:	ELISA (ELISA)
Positive Control:	MCF7 lysate
Negative Control:	Mouse brain lysate
Notes:	Target protein was detected in the positive control sample and not in the negative control sample as expected.
Controls:	• Positive control: MCF7 cell lysate prepared by lysis of cells by freeze-thaw cycles in phosphate buffered saline. • Negative control: Mouse brain lysate prepared by lysis of tissue by freeze-thaw cycles in phosphate buffered saline
Protocol:	• 1. All reagents in the ELISA kit were brought up to room temperature (RT) before use. • 2. 100 µL of standard or sample were added to wells in ELISA plate pre-coated with capture antibody. • 3. All samples and standards were assayed in triplicate. • 4. The plate was covered with sealer (provided in kit) and incubated for 2 hours at 37°C. Unbound material was aspirated but the wells were NOT washed. • 5. 100 µL of Detection Reagent-A Working Solution was added to each well. Plate was covered with sealer (provided in kit) and incubated for 1 hour at 37°C. Unbound material was removed from each well and plate was washed three times with 350 µL of 1x Wash Solution (provided in the kit). After the last wash the plate was inverted and blotted against clean absorbent paper to remove any remaining liquid. • 6. 100 µL of Detection Reagent-B Working Solution was added to each well. Plate was covered with sealer (provided in kit) and incubated for 30 minutes at 37°C. • 7. Unbound material was removed by washing five times with 350 µL of 1x Wash Solution (provided in the kit). After the last wash the plate was inverted and blotted against clean absorbent paper to remove any remaining liquid. • 8. 90 µL of Substrate Solution was added to wells and the plate was covered with a new plate sealer. The plate was gently tapped to ensure mixing and incubated for 25 minutes at 37°C in the dark. • 9. After 25 minutes, when an apparent gradient appeared in the standard wells, the reaction was terminated by adding 50 µL of Stop Solution to each well.

- 10. The optical density (OD value) of each well was read using a microplate reader set to 450 nm.
- 11. The triplicate readings for each sample were averaged and the average zero standard optical density subtracted to yield 'corrected absorbance at 450 nm'. A standard curve was generated by plotting the mean OD value for each standard on the X-axis against the concentration on the Y-axis using Excel. Standard curve was generated by regression analysis with three-parameter logistic.
- 12. An equation (y = -4.6988x^3 + 14.578x^2 + 17.63x + 0.1488) was derived from the standard curve and used to calculate Ki67P concentrations in samples based on their Average Absorbance values.

Experimental Notes: No experimental challenges noted.

Images for Validation report #029832



Validation image no. 1 for Antigen Identified By Monoclonal Antibody Ki-67 (MKI67) ELISA Kit (ABIN415150)

Figure 1: Graph of 'Corrected' OD450 nm plotted for standard curve samples. Standard curve was generated by regression analysis with three-parameter logistic. An equation (y = -4.6988x^3 + 14.578x^2 + 17.63x + 0.1488) was derived from the standard curve and used to calculate KI67P concentrations shown in Figure 2.

Type	Sample, ng/ml	Readings at 450 nm			Avg Reading	Corrected OD450nm	SD	Calculated conc ng/ml
		1	2	3				
Standards	50	1.851	1.868	1.872	1.864	1.733	0.009	50.02
	25	1.067	1.035	1.023	1.042	0.911	0.019	24.74
	12.5	0.648	0.705	0.658	0.670	0.539	0.025	13.16
	6.25	0.397	0.406	0.393	0.399	0.268	0.005	5.82
	3.12	0.257	0.265	0.261	0.261	0.130	0.003	2.68
	1.56	0.202	0.222	0.202	0.209	0.078	0.009	1.60
	0.78	0.184	0.181	0.172	0.179	0.048	0.005	1.03
	0	0.131	0.134	0.129	**0.131**	0.000	0.002	0.15
Spike Controls	0.00	0.128	0.116	0.138	0.127	-0.004	0.009	0.08
	6.25	0.454	0.438	0.463	0.452	0.321	0.010	7.15
Test Samples	MCF7 Extract-PBS	0.852	0.736	0.781	0.723	0.592	0.053	14.72
	MCF7 Extract-PBS 1:4 Diluted	0.292	0.231	0.259	0.261	0.130	0.025	2.67
	Mm Brain Extract-PBS	0.141	0.128	0.130	0.133	0.002	0.006	0.18

Conc of Ki67P in MCF7 (+ve Control) 1:4 diluted: 2.67 x 4 = 11 ng/ml
Conc of Ki67P in MCF7 (+ve Control) undiluted: 15 ng/ml
Conc of Ki67P in Mouse Brain Extract: (-ve Control): 0.18 ng/ml (Below detection range of the kit)

Validation image no. 2 for Antigen Identified By Monoclonal Antibody Ki-67 (MKI67) ELISA Kit (ABIN415150)

Figure 2: Table of absorbance values for standard curve, spike control and samples. Value for Avg Reading is derived from the average reading of three samples. Avg Reading for "0" amount of Standard was subtracted from all Avg Readings to yield "Corrected OD450 nm values" for Standards, spike controls and unknown samples. Standard deviation is included for all samples.