Datasheet for ABIN366268

CD44 ELISA Kit

1 Validation | 1 Image | 2 Publications

Go to Product page

Overview

Quantity:	96 tests
Target:	CD44
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	78-5000 pg/mL
Minimum Detection Limit:	78 pg/mL
Application:	ELISA

Product Details

Purpose:	For the quantitative determination of human cluster of differentiation (CD44) concentrations in serum, plasma, cell culture supernates and tissue homogenates.
Sample Type:	Serum, Plasma, Cell Culture Supernatant, Tissue Homogenate
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This assay has high sensitivity and excellent specificity for detection of human CD44.
Cross-Reactivity (Details):	Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between the target antigen and all analogues for other species. Therefore, cross reaction may still exist.
Sensitivity:	19.5 pg/mL
Components:	• Assay plate (12 × 8 coated Microwells)

Product Details

- Standard (freeze dried)
- Biotin-antibody (100 × concentrate)
- HRP-avidin (100 × concentrate)
- Biotin-antibody Diluent
- HRP-avidin Diluent
- Sample Diluent
- Wash Buffer (25 × concentrate)
- TMB Substrate
- Stop Solution
- Adhesive Strip (for 96 wells)
- Instruction manual

Target Details

Target:	CD44
Alternative Name:	Cluster Of differentiation (CD44) (CD44 Products)
Background:	Synonyms: CDW44, CSPG8, ECMR-III, HCELL, IN, LHR, MC56, MDU2, MDU3, MGC10468, MIC4, MUTCH-I, Pgp1, CD44 antigen|CD44 antigen (homing function and Indian blood group system)|CDW44 antigen|GP90 lymphocyte homin
HGNC:	1681
UniProt:	P16070
Pathways:	Glycosaminoglycan Metabolic Process, Autophagy, Negative Regulation of intrinsic apoptotic Signaling

Application Details

Application Notes:

- The supplier is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
- Samples to be used within 5 days may be stored at 2-8°C, otherwise samples must be stored at -20°C (≤ 1 month) or -80°C (≤ 2 months) to avoid loss of bioactivity and contamination.
- Grossly hemolyzed samples are not suitable for use in this assay.
- If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
- Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
- Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts of certain chemicals.

	• Owing to the possibility of mismatching between antigens from another resource and antibodies used in this supplier's kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by this supplier's products. • Influenced by factors including cell viability, cell number and cell sampling time, samples from cell culture supernatant may not be recognized by the kit. • Fresh samples without long time storage are recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.
Comment:	Detection wavelength: 450 nm Information on standard material: Depending on the antigen to be detected, standards can be either native or recombinant protein. The recombinant proteins are being expressed in CHO cells in most cases. Please inquire for more information. The formulation of auxiliary material in the standard is considered proprietary information, however it does not contain any poisonous substance. Proclin 300 (1:3000) is used as preservative. Information on reagents: In most cases the stop solution provided is 1 N H2SO4. The formulation of wash solution is proprietary information. None of the components contain (sodium) azide, thimerosal, 2-mercaptoethanol (2-ME) or any other poisonous materials. For the sandwich method kits, the sample diluent, antibody diluent, enzyme diluent and standard all contain BSA. Information on antibodies: The antibodies provided in different kits vary in regards to clonality and host. Some antibodies are affinity purified, some are Protein A
Sample Volume:	100 µL
Assay Time:	1 - 4.5 h
Plate:	Pre-coated
Protocol:	This assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for CD44 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any CD44 present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for CD44 is added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) is added to the wells. Following a

	wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of CD44 bound in the initial step. The color development is stopped and the intensity of the color is measured.
Reagent Preparation:	• **Biotin-antibody (1×)** - Centrifuge the vial before opening. Biotin-antibody requires a 100-fold dilution. The suggested dilution is 10µL of Biotin-antibody + 990µL of Biotin-antibody Diluent. • **HRP-avidin (1×)** - Centrifuge the vial before opening. HRP-avidin requires a 100-fold dilution. The suggested dilution is 10µL of HRP-avidin + 990µL of HRP-avidin Diluent. • **Wash Buffer (1×)** - If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20mL of Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500mL of Wash Buffer (1×). • **Standard** - Centrifuge the standard vial at 6000-10000rpm for 30s. Reconstitute the Standard with 1ml of Sample Diluent. Do not substitute other diluents. This reconstitution produces a stock solution. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250µL of Sample Diluent into each tube. Use the stock solution to produce a 2-fold dilution series. Mix each tube thoroughly before the next transfer. The undiluted Standard serves as the high standard. Sample Diluent serves as the zero standard (0ng/mL). Note: • Kindly use graduated containers to prepare the reagent. Please don't prepare the reagent directly in the Diluent vials provided in the kit. • Bring all reagents to room temperature (18-25°C) before use for 30 min. • Prepare fresh standard for each assay. Use within 4 hours and discard after use. • Making serial dilution in the wells directly is not permitted. • Please carefully reconstitute Standards according to the instruction. Avoid foaming and mix gently until the crystals have 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 when pipetting. • It is recommended to use distilled water to prepare reagents and samples. Using contaminated water or container for reagent preparation will influence detection result.
Assay Precision:	Intra-assay precision (precision within an assay): Three samples of known concentration were tested twenty times on one plate to assess precision. Inter-assay precision (precision between assays): Three samples of known concentration were tested in twenty assays to assess precision. • Intra-assay: CV% less than 8% • Inter-assay: CV% less than 10%
Restrictions:	For Research Use only

Handling

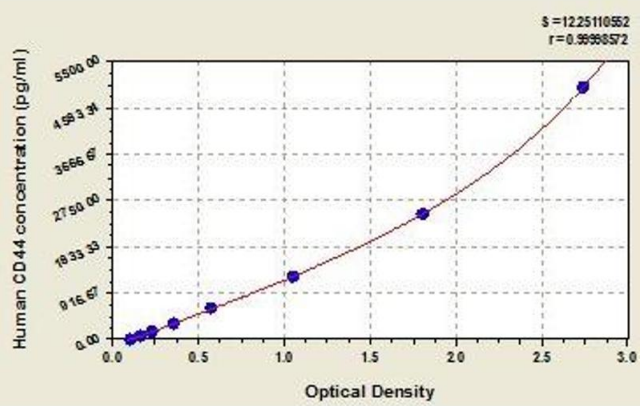
Precaution of Use:	The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face and clothing protection when using this material.
Handling Advice:	• The kit should not be used beyond the expiration date on the kit label. • Do not mix or substitute reagents with those from other lots or sources. • If samples generate values higher than the highest standard, dilute the samples with Sample Diluent and repeat the assay. • Any variation in Sample Diluent, operator, pipetting technique, washing technique, incubation time/temperature and kit age can cause variation in binding. • This assay is designed to eliminate interference by soluble receptors, binding proteins and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.
Storage:	4 °C/-20 °C
Storage Comment:	For unopened kit: All the reagents should be kept according to the labels on vials.
Expiry Date:	6 months

Publications

Product cited in:	Zhou, Sui, Mo, Sun: "Multifunctional and biomimetic fish collagen/bioactive glass nanofibers: fabrication, antibacterial activity and inducing skin regeneration in vitro and in vivo." in: **International journal of nanomedicine**, Vol. 12, pp. 3495-3507, (2017) (PubMed). Mittal, Kumar: "A new, bioactive, antibacterial-eluting, composite graft for infection-free wound healing." in: **Wound repair and regeneration : official publication of the Wound Healing Society [and] the European Tissue Repair Society**, Vol. 22, Issue 4, pp. 527-36, (2015) (PubMed). Liu, Huang, Chen, Zhang, Li, Wang, Ge, Wang, Zhang: "Mechanical stretch promotes matrix metalloproteinase-2 and prolyl-4-hydroxylase ?1 production in human aortic smooth muscle cells via Akt-p38 MAPK-JNK signaling." in: **The international journal of biochemistry & cell biology**, Vol. 62, pp. 15-23, (2015) (PubMed). Guo, Wang, Zhou, Wu, Ma, Liu, Huang, Qin: "Lentiviral Vector-Mediated FoxO1 Overexpression Inhibits Extracellular Matrix Protein Secretion under High Glucose Conditions in Mesangial Cells." in: **Journal of cellular biochemistry**, (2015) (PubMed). Stefanovic, Cukuranovic, Dolicanin, Cukuranovic, Stojnev, Bogdanovic, Rajic, Kocic: "Placental

growth factor and placental protein 13 in patients with Balkan endemic nephropathy, a worldwide disease." in: **Renal failure**, pp. 1-4, (2015) (PubMed).

Validation report #029790 for ELISA (ELISA)



ELISA

Image 1. Typical standard curve

Validation report #029852 for ELISA (ELISA)

Successfully validated (ELISA (ELISA))

by Affina Biotechnologies, Inc

Report Number: 029852

Date: Apr 07 2016

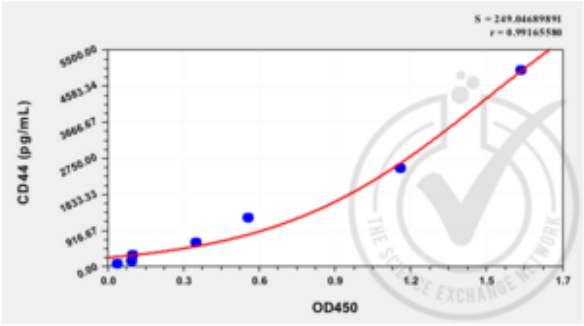
Target:	Human CD44 (cluster Of differentiation)
Lot Number:	V15182623
Method validated:	ELISA (ELISA)
Positive Control:	Human pooled serum (Biochemed, 750-NS-FI-POM, Lot#BC033016HSPMG)
Negative Control:	Chicken plasma (Biochemed, Lot#BC03316CSPMG)
Notes:	CD44 was clearly detected in the positive sample serum. While calculated values for the chicken serum are high the reading of the chicken serum in this ELISA could not be distinguished from the blank and thus are actually very low. Spike recovery was >100% indicating that chicken serum did not interfere with the assay at 50-fold dilution.
Controls:	• Positive control: Human pooled serum (Biochemed, 750-NS-FI-POM, Lot#BC033016HSPMG) • Negative control: Chicken plasma (Biochemed, Lot#BC03316CSPMG) • Standard curve: 0, 75, 156, 312, 625, 1250, 2500 and 5000 pg/mL CD44 provided in the ELISA kit • Spike control: 2500 pg/mL standard premixed with chicken plasma in a 1:1 ratio
Protocol:	• 1. 100 µL of standard and samples were added 96-well strip plates provided in the kit. All samples and standards were assayed in duplicate. • 2. Removed liquid from wells without washing • 3. 100 µl /well of Biotin-antibody conjugate added to each well and incubated at 37°C for 1 hr. Washed 3 times (200 µL/well, 2min soak), • 4. 100 µL of HRP conjugate was added and contents in the wells were mixed. • 5. The microplate was covered and incubated at 37°C for 1 hr. • 6. Plate contents were discarded and wells were washed 5 times with 200 µl of 1x wash solution. • 7. 90 µl of TMB susbtrate was added to each well. The plate was covered and incubated at 37°C for 10 min. • 8. 50 µl of the Stop Solution was added per well. • 9. The optical density (OD value) of each well was read immediately using a microplate reader set to 450 nm. • 10. The duplicate readings for each sample were averaged and the average zero standard optical density subtracted. The corrected average-value was tabulated as Average

Absorbance. 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 CurveExpert 1.4. A logistic fit through the points on the graph was used to calculate concentrations.

- 11. The Analyze feature of CurveFit expert was used to calculate CD44 concentrations of the samples based on their Average Absorbance values.

Experimental Notes:	- The concentration of CD44 in human and chicken plasmas was measured according to the manufacturers directions.

Images for Validation report #029852



Validation image no. 1 for CD44 (CD44) ELISA Kit (ABIN366268)

Figure 1: Graph of corrected-average absorbance (OD 450 nm) readings plotted for standard curve samples.

Type	Sample pg/ml	Reading-1	Reading-2	Avg Reading	Avg Absorbance	SD	Calculated Conc
Standard Curve	5000	1.647	1.664	1.655	1.586	0.012718	4974
	2500	1.177	1.207	1.192	1.123	0.021028	2634
	1250	0.684	0.537	0.610	0.541	0.104035	821
	625	0.426	0.390	0.408	0.339	0.025403	519
	312.5	0.187	0.144	0.165	0.096	0.030157	293
	156.25	0.150	0.173	0.161	0.092	0.016405	290
	78.125	0.113	0.099	0.106	0.037	0.009514	255
	0.000	0.078	0.060	0.069	0.000	0.012654	233
Spike Control	1250	0.920	0.951	0.936	0.867	0.021689	1638
Positive Control	Human serum (50-dilute)	0.369	0.376	0.373	0.308	0.004962	24150
Negative control	Chicken Plasma (50-dilute)	0.049	0.048	0.048	0.000	0.001132	11400

Validation image no. 2 for CD44 (CD44) ELISA Kit (ABIN366268)

Figure 2: Table of absorbance readings (OD 450 nm) for standard curve, spike controls and unknown control samples. Value for Average Reading is derived from the average of two readings (OD 450nm). The Average Reading for blank sample (no conjugate added) was subtracted from all Average Readings to yield Average Absorbance values for Standards, spike controls and control samples. Standard deviation is included for all samples. The values were generated from the logistic fit to the results (CurveExpert 1.4) for the standard curve and used to calculate CD44 concentrations shown in the Table.