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colorimetric sandwich ELISA kit datasheet

For the quantitative detection of human CD20 in cell culture supernatants and cell lysates.

general information

Catalogue Number	KE00082
Product Name	CD20 ELISA Kit
Species cross-reactivity	Human CD20
Range (calibration Range)	0.78 - 50 ng/mL
Tested applications	Quantification ELISA

database links

Entrez Gene	**931** (Human)
SwissProt	**P11836** (Human)

kit components & storage

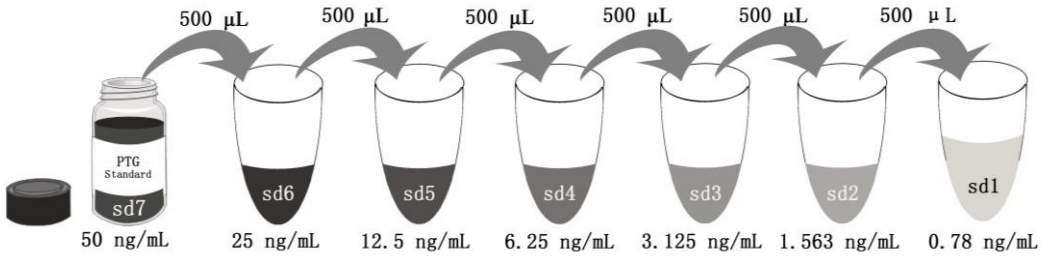
Microplate - antibody coated 96-well Microplate (8 wells ×12 strips)	1 plate	Store at -20°C for six months
Standard – 100 ng/bottle; lyophilized*	2 bottles	Store at -20°C for six months
Detection antibody (100X) - 150 µL/vial	1 vial	Store at 2-8°C for six months
HRP-conjugated antibody (100X) - 150 µL/vial	1 vial	Store at 2-8°C for six months
Sample Diluent PT 5-ef - 30 mL/bottle	1 bottle	Store at 2-8°C for six months
Detection Diluent - 30 mL/bottle	1 bottle	Store at 2-8°C for six months
Wash Buffer Concentrate (20X) - 30 mL/bottle	1 bottle	Store at 2-8°C for six months
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	Store at 2-8°C for six months
Stop Solution - 12 mL/bottle	1 bottle	Store at 2-8°C for six months
Plate Cover Seals	3 pieces	

NB: Do not use the kit after the expiration date.

Sample Diluent PT 5-ef is for Standard, cell culture supernatants and cell lysate samples.

Detection Diluent is for Detection antibody and HRP-conjugated antibody.

*Add 2 mL Sample Diluent PT 5-ef in Standard, This reconstitution gives a stock solution of 50 ng/mL.



Add # µL of Standard diluted in the previous step	—	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
# µL of Sample Diluent PT 5-ef	2000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

product description

KE00082 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The CD20 ELISA kit is to be used to detect and quantify protein levels of endogenous CD20. The assay recognizes human CD20. A polyclonal antibody specific for CD20 has been pre-coated onto the microwells. The CD20 protein in samples is captured by the coated antibody after incubation. Following extensive washing, a monoclonal antibody specific for CD20 is added to detect the captured CD20 protein. For signal development, horseradish peroxidase (HRP)-conjugated Anti-mouse antibody is added, followed by Tetramethyl-benzidine (TMB) reagent. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450nm.

background

CD20 is a 33-37 kDa transmembrane phosphoprotein. CD20 is a B-lymphocyte surface molecule that is widely expressed during B-cell ontogeny, from early pre-B-cell developmental stages until final differentiation into plasma cells. CD20 functions as calcium-permeable cation channel. It is involved in the regulation of B-cell activation and proliferation. CD20 serves as a useful target for antibody-mediated therapeutic depletion of B-cells.

sample preparation

The cell culture supernatants and cell lysates. may require proper dilution to fall within the range of the assay. A range of dilutions like 1:2, 1:4 is suggested according to the individual samples.

safety notes

This product is sold for lab research and development use ONLY and not for use in humans or animals.
Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

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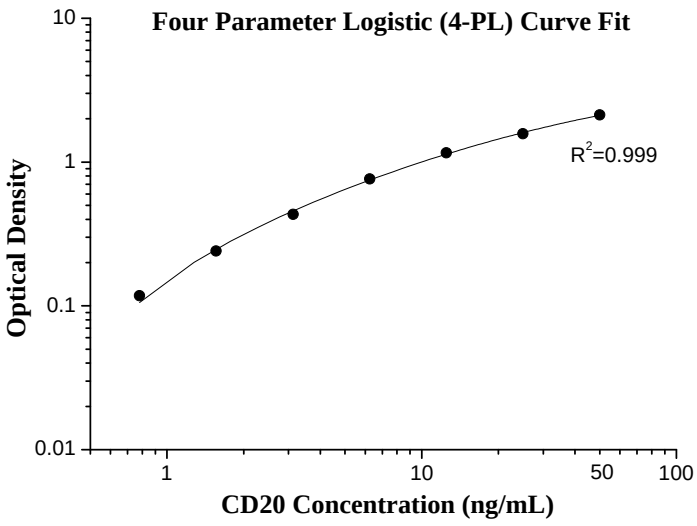
assay procedure summary

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	**120 min**	4 times	Cover Wells
2	Diluent Antibody Solution	100 µL	60 min	4 times	Cover Wells
3	Diluent HRP Solution	100 µL	40 min	4 times	Cover Wells
4	TMB Substrate	100 µL	15-30 min	Do not wash	Incubate in the dark at 37 °C
5	Stop Solution	100 µL	0 min	Do not wash	-
6	Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes.				

typical data

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(ng/mL)	O.D	Average	Corrected
0	0.016 0.016	0.016	—
0.78	0.136 0.131	0.1335	0.1175
1.56	0.269 0.245	0.257	0.241
3.13	0.45 0.449	0.4495	0.4335
6.25	0.789 0.77	0.7795	0.7635
12.5	1.19 1.163	1.1765	1.1605
25	1.587 1.592	1.5895	1.5735
50	2.108 2.176	2.142	2.126

precision

Intra-assay Precision (Precision within an assay) Three samples of known concentration were tested 20 times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) Three samples of known concentration were tested in 24 separate assays to assess inter-assay precision.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	24	24	24
Mean (ng/ml)	38.84	10.59	2.58	42.34	10.04	2.50
SD	2.40	0.39	0.11	4.08	0.67	0.15
CV%	6.2	3.7	4.1	9.6	6.6	6.1

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recovery

The recovery of CD20 spiked to three different levels in four samples throughout the range of the assay in vrious matrices was evaluated.

Sample Type		Average % of Expected	Range(%)
Cell culture supernatants	1:2	97	83-119
	1:4	99	83-122
Cell lysates	1:2	102	75-121
	1:4	99	86-120

sample values

Raji human Burkitt's lymphoma cells (5 x 10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal bovine serum, 2mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. 1 x 10^6 cells/mL were were lysed used by RIPA buffer, and the supernate was assayed and measured 1118 ng/mL.

sensitivity

The minimum detectable dose of human CD20 is 0.33 ng/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

linearity

To assess the linearity of the assay, three samples were spiked with high concentrations of CD20 in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay.
(The samples were initially diluted 1:3)

		Cell culture supernatants	Cell lysates
1:2	Average% of Expected	100	100
	Range(%)	94-111	88-112
1:4	Average% of Expected	109	100
	Range(%)	93-127	84-115
1:8	Average% of Expected	99	107
	Range(%)	93-106	83-114
1:16	Average% of Expected	101	101
	Range(%)	93-107	90-112

references

1. Tedder TF, et al. CD20: a regulator of cell-cycle progression of B lymphocytes. Immunol Today. 15(9):450-4 (1994).
2. Cragg MS, et al. The biology of CD20 and its potential as a target for mAb therapy. Curr Dir Autoimmun. 8:140-74 (2005).