

colorimetric sandwich ELISA kit datasheet

For the quantitative detection of human BIRC5 in serum, plasma, cell culture supernatants and urine.

general information

Catalogue Number	KE00043
Product Name	BIRC5 ELISA Kit
Species cross-reactivity	Human BIRC5
Range (calibration Range)	62.5 - 4000 pg/mL
Tested applications	Quantification ELISA

database links

Entrez Gene	332 (Human)
SwissProt	O15392 (Human)

kit components & storage

Microplate - antibody coated 96-well Microplate (8 wells × 12 strips)	1 plate	Store at -20°C for six months
Standard - 8000 pg/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 1-dg - 30 mL/bottle. For serum, plasma and urine samples	1 bottle	Store at 2-8°C for six months
Sample Diluent PT 1-ef - 30 mL/bottle. For cell culture supernatants	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	

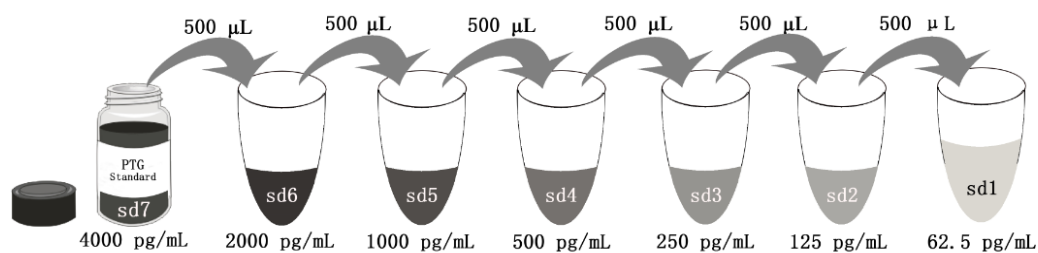
NB: Do not use the kit after the expiration date.

Sample Diluent PT 1-dg is for Standard, serum, plasma and urine samples.

Sample Diluent PT 1-ef is for Standard and cell culture supernatants.

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

*Add 2 mL Sample Diluent PT 1-dg or PT 1-ef in Standard, This reconstitution gives a stock solution of 4000 pg/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 1-dg or PT 1-ef	2000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

product description

KE00043 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The BIRC5 ELISA kit is to be used to detect and quantify protein levels of endogenous BIRC5. The assay recognizes human BIRC5. A polyclonal antibody specific for BIRC5 has been pre-coated onto the microwells. The BIRC5 protein in samples is captured by the coated antibody after incubation. Following extensive washing, a monoclonal antibody specific for BIRC5 is added to detect the captured BIRC5 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

Survivin, also called BIRC5, is a unique member of the inhibitor of apoptosis (IAP) protein family. Survivin is a 16 kDa anti-apoptotic protein highly expressed during fetal development and cancer cell malignancy, but is completely absent in terminally differentiated cells. The differential expression of survivin in cancer versus normal tissues makes it a useful tool in cancer diagnosis and a promising therapeutic target. Survivin expression is also highly regulated by the cell cycle and is only expressed in the G2-M phase. It is known that survivin localizes to the mitotic spindle by interaction with tubulin during mitosis and may play a contributing role in regulating mitosis. Disruption of survivin-microtubule interactions results in loss of survivin's anti-apoptosis function and increased caspase-3 activity, a mechanism involved in cell death, during mitosis. It also is a direct target gene of the Wnt pathway and is upregulated by beta-catenin.

sample preparation

The serum or plasma samples 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

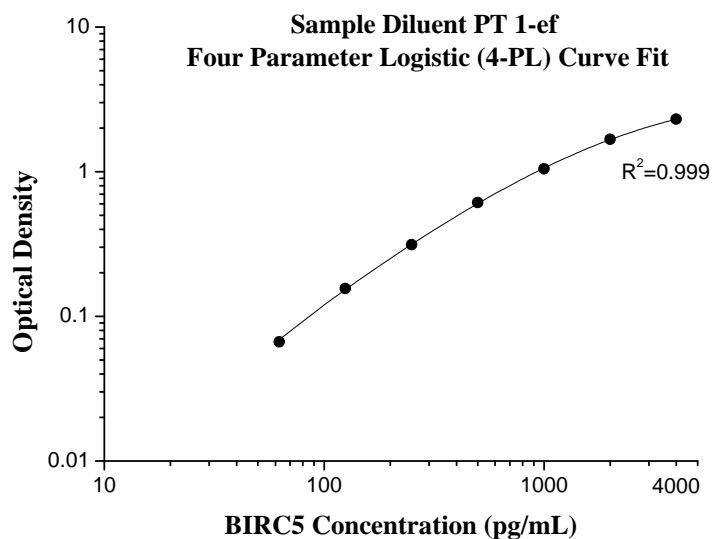
This product is sold for lab research and development use ONLY and not for use in humans or animals.
Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

assay procedure summary

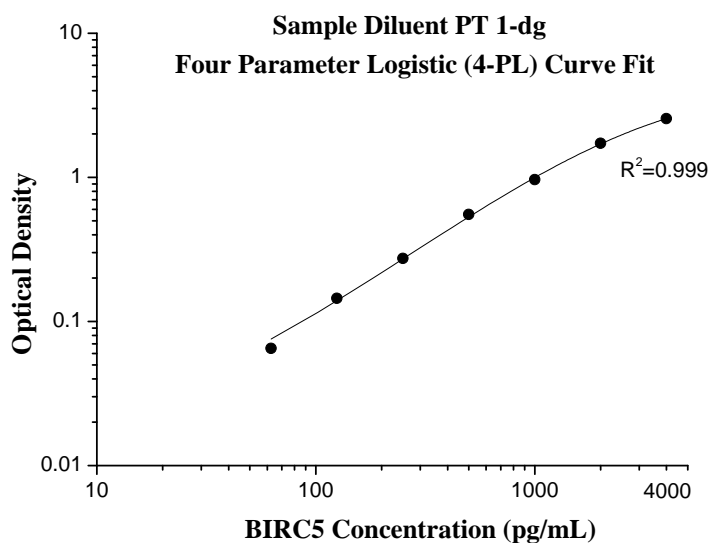
Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	60 min	4 times	Cover Wells
2	Diluent Antibody Solution	100 µL	60 min	4 times	Cover Wells
3	Diluent HRP Solution	100 µL	40 min	4 times	Cover Wells
4	TMB Substrate	100 µL	15-30 min	Do not wash	Incubate in the dark at 37°C
5	Stop Solution	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.



(pg/mL)	O.D	Average	Corrected
0	0.076	0.0825	—
	0.089		
62.5	0.146	0.149	0.0665
	0.152		
125	0.228	0.238	0.1555
	0.248		
250	0.399	0.395	0.3125
	0.391		
500	0.683	0.694	0.6115
	0.705		
1000	1.145	1.1285	1.0465
	1.112		
2000	1.766	1.7575	1.675
	1.749		
4000	2.351	2.3875	2.305
	2.424		



(pg/mL)	O.D	Average	Corrected
0	0.078	0.081	—
	0.084		
62.5	0.14	0.146	0.065
	0.152		
125	0.212	0.226	0.145
	0.24		
250	0.344	0.355	0.274
	0.366		
500	0.592	0.6335	0.5525
	0.675		
1000	1.001	1.044	0.936
	1.087		
2000	1.776	1.805	1.724
	1.834		
4000	2.599	2.6375	2.5565
	2.676		

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.

Sample	Intra-assay Precision			Inter-assay Precision		
	1	2	3	1	2	3
n	20	20	20	24	24	24
Mean (pg/ml)	2289.5	1216.8	293.8	2495.0	1253.5	307.6
SD	110.5	35.7	15.9	159.3	77.4	24.8
CV%	4.8	2.9	5.4	6.4	6.2	8.1

recovery

The recovery of BIRC5 spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Sample Type		Average % of Expected	Range(%)
Citrate plasma	1:2	94	82-114
	1:4	100	88-125
Cell culture supernatants	1:2	110	94-130
	1:4	109	91-125
Urine	1:2	101	83-118
	1:4	99	80-119

sensitivity

The minimum detectable dose of human BIRC5 is 35 pg/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 BIRC5 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 1:1)

		Citrate plasma (Sample Diluent PT 1-dg)	Cell culture supernatants (Sample Diluent PT 1-ef)	Urine (Sample Diluent PT 1-dg)
1:2	Average% of Expected	104	114	111
	Range(%)	101-107	105-129	99-123
1:4	Average% of Expected	112	116	106
	Range(%)	108-116	112-124	104-109
1:8	Average% of Expected	100	105	100
	Range(%)	97-102	102-109	100-112
1:16	Average% of Expected	93	99	97
	Range(%)	89-97	98-99	97-116

references

1. Reed. et al. (1999). Nature Cell Biol. 1: 199-200.
2. Sah N.K. et al. (2006). Cancer Letters. 244: 164-71.
3. Li F. et al. (1998). Nature. 396: 580-4.
4. Olie RA. et al. (2000). Cancer Res. 60: 2805-9.