ab326749 – Human CYR61 SimpleStep ELISA® Kit, Chemiluminescent

For the quantitative measurement of CYR61 in human serum, plasma (citrate), cell culture supernatant, saliva, urine, milk, cell extract, and tissue extract.
For research use only - not intended for diagnostic use.
Patent pending.

For overview, typical data and additional information please visit: www.abcam.com/ab326749

Storage and Stability: Store kit at 2-8°C immediately upon receipt. Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Standard Preparation and Reagent preparation sections.

Limitations: All data, except typical standard curve, sensitivity, and native signal linearity for serum, plasma (citrate) and urine were collected using the colorimetric version of this kit (ab238267).

Materials Supplied

Item	Quantity 1 x 96 tests	Storage Condition
Human CYR61 Capture Antibody 10X	600 µL	+4°C
Human CYR61 Detector Antibody 10X	600 µL	+4°C
Human CYR61 Lyophilized Recombinant Protein	2 Vials	+4°C
Antibody Diluent 5BI	6 mL	+4°C
Cell Extraction Buffer PTR 5X	10 mL	+4°C
Cell Extraction Enhancer Solution 50X	1 mL	+4°C
Sample Diluent 25BP	20 mL	+4°C
Sample Diluent NS	50 mL	+4°C
Wash Buffer PT 10X	20 mL	+4°C
ChemiHRP Reagent A	3 mL	+4°C
ChemiHRP Reagent B	3 mL	+4°C
SimpleStep Pre-Coated Black 96-Well Microplate	96 Wells	+4°C
Plate Seal	1	+4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

Luminometer capable of reading strip plates with the following settings: 0.5-1 second/well read time; summation mode (all wavelengths).
Method for determining protein concentration (BCA assay recommended).
Deionized water.
Multi- and single-channel pipettes.
Tubes for standard dilution.
Orbital microplate shaker for all incubation steps: capable of 750 rpm shaking speed.
Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

Reagent Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.

Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations.

Sample Diluent 25BP may contain precipitate, this is normal. If precipitate is not dissolved by gentle mixing, the precipitate may be dissolved by gentle warming and mixing at 37°C for 10 minutes. If precipitate remains, gently spin down and avoid visible precipitates when pipetting.

The provided Cell Extraction Enhancer Solution 50X may precipitate when stored at + 4°C. To dissolve, warm briefly at + 37°C and mix gently. The Cell Extraction Enhancer Solution 50X can be stored at room temperature to avoid precipitation.

1X Wash Buffer PT: Prepare 1X Wash Buffer PT by diluting Wash Buffer PT 10X with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL Wash Buffer PT 10X with 45 mL deionized water. Mix thoroughly and gently.

1X Cell Extraction Buffer PTR (For cell and tissue extracts only): Prepare 1X Cell Extraction Buffer PTR by diluting Cell Extraction Buffer PTR 5X and 50X Cell Extraction Enhancer Solution to 1X with deionized water. To make 10 mL 1X Cell Extraction Buffer PTR combine 7.8 mL deionized water, 2 mL Cell Extraction Buffer PTR 5X and 200 µL Cell Extraction Enhancer Solution 50X. Mix thoroughly and gently. If required protease inhibitors can be added.

Antibody Cocktail: Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent 5BI. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent 5BI. Mix thoroughly and gently.

Lumi HRP Development Solution: Just prior to use, prepare Lumi HRP Development Solution by mixing equal volume of the ChemiHRP Reagent A and the ChemiHRP Reagent B. To make 3 mL of the Lumi HRP Development Solution combine 1.5 mL of ChemiHRP Reagent A and 1.5 mL of ChemiHRP Reagent B. Mix thoroughly and gently by inversion or slow pipetting (Avoid shaking or vortexing). Protect the prepared solution from light until use.

Standard Preparation

Always prepare a fresh set of standards for every use. Discard working standard dilutions after use as they do not store well. The following section describes the preparation of a standard curve for duplicate measurements (recommended).

For serum and plasma (citrate) samples measurements:

1. Reconstitute the CYR61 standard sample by adding the volume of Sample Diluent 25BP indicated on the protein vial label. Hold at room temperature for 10 minutes. Mix thoroughly and gently. This is the 4,000 pg/mL **Stock Standard** Solution.
2. Label seven tubes, Standards 1– 7.
3. Add 150 µL of Sample Diluent 25BP into numbers 2-7.

4. Use the **Stock Standard** to prepare the following dilution series. Standard #7 contains no protein and is the Blank control:

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	**Stock Standard**	225	0	4,000	4,000
2	Standard#1	75	150	4,000	1,333.3
3	Standard#2	75	150	1,333.3	444.4
4	Standard#3	75	150	444.4	148.2
5	Standard#4	75	150	148.2	49.4
6	Standard#5	75	150	49.4	16.5
7	Standard#6	0	150	0	0

For cell culture supernatant, milk, urine, and saliva samples measurements:

1. Reconstitute the CYR61 standard sample by adding the volume of Sample Diluent NS indicated on the protein vial label. Hold at room temperature for 10 minutes. Mix thoroughly and gently. This is the 4,000 pg/mL **Stock Standard** Solution.
2. Label seven tubes, Standards 1– 7.
3. Add 150 µL of Sample Diluent NS into numbers 2-7.
4. Use the **Stock Standard** to prepare the following dilution series. Standard #7 contains no protein and is the Blank control:

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	**Stock Standard**	225	0	4,000	4,000
2	Standard#1	75	150	4,000	1,333.3
3	Standard#2	75	150	1,333.3	444.4
4	Standard#3	75	150	444.4	148.2
5	Standard#4	75	150	148.2	49.4
6	Standard#5	75	150	49.4	16.5
7	Standard#6	0	150	0	0

For cell extract and tissue extract samples measurements:

1. Reconstitute the CYR61 standard sample by adding the volume of **1X Cell Extraction Buffer PTR** indicated on the protein vial label. Hold at room temperature for 10 minutes. Mix thoroughly and gently. This is the 4,000 pg/mL **Stock Standard** Solution.
2. Label eight tubes, Standards 1– 8.
3. Add 150 µL of **1X Cell Extraction Buffer PTR** into numbers 2-8.
4. Use the **Stock Standard** to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	**Stock Standard**	225	0	4,000	4,000
2	Standard#1	75	150	4,000	1,333.3
3	Standard#2	75	150	1,333.3	444.4
4	Standard#3	75	150	444.4	148.2
5	Standard#4	75	150	148.2	49.4
6	Standard#5	75	150	49.4	16.5
7	Standard#6	75	150	16.5	5.5
8	Standard#7	75	150	0	0

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	**Range**
Serum	25 – 100%
Plasma - Citrate	25 – 100%
Breast Milk (de-fatted)	6.25 – 100%
Urine	50 - 100%
Saliva	6.25 – 100%
HeLa Cell Culture Supernatant	1.56 – 25%
MDA-MB-453S Cell Culture Supernatant	1.56 – 25%
HeLa Cell Extract	7.8 – 250 µg/mL
MDA-MB-453S Cell Extract	15.6 – 500 µg/mL
Human Liver Tissue Extract*	≤300 µg/mL

* Based on spiked sample

Serum Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. Assay, or dilute samples into Sample

Diluent 25BP and assay. Store un-diluted serum at -20ºC or below. Avoid repeated freeze-thaw cycles.

Plasma Collect plasma using citrate. Centrifuge samples at 2,000 x g for 10 minutes. Assay, or dilute samples into Sample Diluent 25BP and assay. Store un-diluted plasma samples at -20ºC or below for up to 3 months. Avoid repeated freeze-thaw cycles. Note: This kit is incompatible with plasma (EDTA, heparin) samples.

Cell Culture Supernatants Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants. Dilute samples at least 1:4 into Sample Diluent NS and assay. Store un-diluted samples at -20ºC or below. Avoid repeated freeze-thaw cycles.

Urine Centrifuge urine at 2,000 x g for 10 minutes to remove debris. Assay, or dilute samples into Sample Diluent NS and assay. Store un-diluted urine samples at -20ºC or below. Avoid repeated freeze-thaw cycles.

Saliva Centrifuge saliva at 800 x g for 10 minutes to remove debris. Collect supernatants. Assay, or dilute samples into Sample Diluent NS and assay. Store un-diluted samples at -20ºC or below. Avoid repeated freeze-thaw cycles.

Milk De-fat milk samples as follows. Centrifuge milk samples at 500 x g for 15 minutes at 4ºC and collect the aqueous fraction using syringe attached to needle. Centrifuge the aqueous fraction at 3,000 x g for 15 minutes at 4ºC and collect the final aqueous fraction (de-fatted milk) using syringe attached to needle. Assay, or dilute samples into Sample Diluent NS and assay. Store un-diluted de-fatted milk at -20ºC or below. Avoid repeated freeze-thaw cycles.

Preparation of extracts from cell pellets Collect non-adherent cells by centrifugation or scrape to collect adherent cells from the culture flask. Typical centrifugation conditions for cells are 500 x g for 5 minutes at 4ºC. Rinse cells twice with PBS. Solubilize pellet at $2x10^7$ cell/mL in chilled 1X Cell Extraction Buffer PTR. Incubate on ice for 20 minutes. Centrifuge at 18,000 x g for 20 minutes at 4ºC. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80ºC. The sample protein concentration in the extract may be quantified using a protein assay. Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

Preparation of extracts from adherent cells by direct lysis (alternative protocol) Remove growth media and rinse adherent cells 2 times in PBS. Solubilize the cells by addition of chilled 1X Cell Extraction Buffer PTR directly to the plate (use 750 µL - 1.5 mL 1X Cell Extraction Buffer PTR per confluent 15 cm diameter plate). Scrape the cells into a microfuge tube and incubate the lysate on ice for 15 minutes. Centrifuge at 18,000 x g for 20 minutes at 4ºC. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80ºC. The sample protein concentration in the extract may be quantified using a protein assay. Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

Preparation of extracts from tissue homogenates Tissue lysates are typically prepared by homogenization of tissue that is first minced and thoroughly rinsed in PBS to remove blood (dounce homogenizer recommended). Homogenize 100 to 200 mg of wet tissue in 500 µL – 1 mL of chilled 1X Cell Extraction Buffer PTR. For lower amounts of tissue adjust volumes accordingly. Incubate on ice for 20 minutes. Centrifuge at 18,000 x g for 20 minutes at 4ºC. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80ºC. The sample protein concentration in the extract may be quantified using a protein assay. Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

Plate Preparation

The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.

Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4ºC.

For each assay performed, a minimum of two wells must be used as the zero control.

For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

Differences in well absorbance or "edge effects" have not been observed with this assay.

Assay Procedure

Equilibrate all materials and prepared reagents to room temperature prior to use.

We recommend that you assay all standards, controls and samples in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4ºC storage.
3. Add 50 µL of all sample or standard to appropriate wells.
4. Add 50 µL of the Antibody Cocktail to each well.
5. Seal the plate and incubate for 30 minutes at room temperature on a plate shaker set to 750 rpm.
6. Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Wash Buffer PT should remain in wells for at least 30 seconds. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and tap gently against clean paper towels to remove excess liquid.
7. Add 50 µL of prepared Lumi HRP Development Solution to each well and incubate for 1 minute in the dark on a plate shaker set to 750 rpm. Further optimization of incubation time vs signal strength can be performed if needed. Avoid introducing bubbles into the wells.
8. Measure the produced light of each well using a microplate luminometer with the following settings: 0.5-1 second/well read time in summation mode (all wavelengths). Relative light unit (RLU) readings may vary between luminometer models. It is recommended to configure instrument settings according to the manufacturer's specifications. Note: Relative light unit (RLU) values may change over the course of the 15-minute reading window.
9. Analyze the data as described below.

Mode:	Luminescence
Instrument settings:	Endpoint
Detection Mode:	All wavelengths
Read Time:	0.5-1 sec
Read:	Top

Note For microplate readers with Pre-Read Optimization option, the Read Height as well as Microplate Optimization is recommended before the first read.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
https://www.abcam.com/en-us/technical-resources/guides/elisa-guide

Technical Support

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For all technical or commercial enquiries please go to:
https://www.abcam.com/en-us/contact-us
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https://www.abcam.co.jp/contact-us (Japan)

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Additional information

ASSAY SPECIFICITY

This kit is designed for the quantification of human CYR61.

The standard protein in this kit is full length mature human CYR61.

Native signal was detected in serum, plasma (citrate), cell culture supernatant, urine, saliva, milk, and cell extract sample types.

Spiked protein experiments were used to validate serum, plasma (citrate), urine, and tissue extract sample types.

CSF samples have not been tested with this kit.

This kit is incompatible with plasma (EDTA) and plasma (heparin) samples.

INTERFERENCE

Recombinant human ITGB1 and human ITGB3 proteins were prepared at 50 ng/mL and 1 ng/mL and tested for interference with 1 ng/mL of recombinant CYR61 protein. No interference with was observed.

SPECIES REACTIVITY

Other species reactivity was determined by measuring neat serum samples of various species, interpolating the protein concentrations from the human standard curve, and expressing the interpolated concentrations as a percentage of the protein concentration in human serum assayed at the same dilution.

Species	% Reactivity
Mouse	100
Bovine	<3%

Other species reactivity not determined.

CALCULATION

- Preconfigured protocols are available when using SoftMax Pro software from Molecular Devices.
- Calculate the average chemiluminescence value for the blank control (zero) standards. Subtract the average blank control standard chemiluminescence value from all other chemiluminescence values.
- Create a standard curve by plotting the average blank control subtracted chemiluminescence value for each standard concentration (y-axis) against the target protein concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.
- Note: Most chemiluminescence reader software or graphing software will plot these values and fit a curve to the data. A four-parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4-parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.
- Determine the concentration of the target protein in the sample by interpolating the blank control subtracted chemiluminescence values against the standard curve. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.
- Samples generating chemiluminescence values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at chemiluminescence values less than that of the lowest standard should be retested in a less dilute form.

TYPICAL DATA

Typical standard curve – data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

Standard Curve Measurements			
Concentration (pg/mL)	RLU		Mean RLU
	1	2	
0.0	13,084	13,365	13,225
16.5	21,255	20,806	21,031
49.4	28,438	27,212	27,825
148.1	55,949	60,915	58,432
444.4	144,750	139,960	142,355
1,333.3	407,620	397,920	402,770
4,000	1,160,000	1,166,400	1,163,200

Table 1. Example of human CYR61 standard curve in Sample Diluent 25BP. The CYR61 standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

Standard Curve Measurements			
Concentration (pg/mL)	RLU		Mean RLU
	1	2	
0.0	15,427	14,735	15,081
16.5	32,793	31,793	32,293
49.4	50,220	52,601	51,411
148.1	118,470	123,840	121,155
444.4	393,230	393,230	393,230
1,333.3	1,138,100	1,133,000	1,135,550
4,000	2,700,800	2,704,800	2,702,800

Table 2. Example of human CYR61 standard curve in Sample Diluent NS. The CYR61 standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

Standard Curve Measurements			
Concentration (pg/mL)	RLU		Mean RLU
	1	2	
0.0	13,547	14,531	14,039
5.5	20,418	21,143	20,781
16.5	38,995	40,754	39,875
49.4	73,040	74,173	73,607
148.1	195,990	204,190	200,090
444.4	607,870	601,680	604,775
1,333.3	1,307,300	1,364,400	1,335,850
4,000	2,661,700	2,647,900	2,654,800

Table 3. Example of human CYR61 standard curve in 1X Cell Extraction Buffer PTR. The CYR61 standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

TYPICAL SAMPLE VALUES

Sensitivity:

The minimal detectable dose (MDD) was determined by calculating the mean of zero standard replicates and adding 2 standard deviations then extrapolating the corresponding concentration.

Sample Diluent Buffer	N=	Minimal Detectable Dose
Sample Diluent 25BP	16	6.11 pg/mL
Sample Diluent NS	16	5.00 pg/mL
1X Cell Extraction Buffer PTR	16	2.82 pg/mL

Recovery

Three concentrations of CYR61 were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
100% Serum	112	108 – 115
100% Plasma - Citrate	92	86 – 96
25% Breast Milk (de-fatted)	95	93 – 96
100% Urine	107	103 – 113
25% Saliva	114	110 – 119
2.5% HeLa Cell Culture Supernatant	100	94 – 103
10% MDA-MB-453S Cell Culture Supernatant	110	106 – 115
30 µg/mL HeLa Cell Extract	83	80 – 85
30 µg/mL MDA-MB-453S Cell Extract	90	82 – 96
300 µg/mL Human Liver Tissue Extract	84	80 – 88
100% Cell Culture Media*	91	89 – 96

*Media is DMEM containing 10% fetal bovine serum

Linearity of Dilution

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Recombinant CYR61 was spiked into the following biological samples and diluted in a 2-fold dilution series in Sample Diluent 25BP for serum and plasma, Sample Diluent NS for urine, and 1X Cell Extraction Buffer PTR for liver tissue extract.

Dilution Factor	Interpolated value	100% Serum	100% Plasma (Citrate)	100% Urine	300 µg/mL Liver Tissue Extract
Undiluted	pg/mL	1,800	1,506	860.1	464.1
	% Expected value	100	100	100	100
2	pg/mL	981.4	828.3	446.1	219.6
	% Expected value	109	110	104	95
4	pg/mL	467.4	414.8	206.8	102.8
	% Expected value	104	110	96	89
8	pg/mL	222.8	212.6	106.3	52.41
	% Expected value	99	113	99	90
16	pg/mL	102.6	107.9	50.85	24.52
	% Expected value	91	115	95	85

Native CYR61 was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent 25BP for serum and plasma and Sample Diluent NS for urine, saliva, and milk.

Dilution Factor	Interpolated value	100% Serum	100% Plasma (Citrate)	100% Urine	100% Saliva	100% Milk
Undiluted	pg/mL	167.3	209.2	35.7	883.9	1,582
	% Expected value	100.0	100.0	100	100	100
2	pg/mL	87.0	123.9	15.0	386.9	822.4
	% Expected value	104.0	118.0	84	88	104
4	pg/mL	45.7	43.0	ND	198.1	346.9
	% Expected value	109.0	82.0	ND	90	88
8	pg/mL	ND	ND	ND	96.64	160.1
	% Expected value	ND	ND	ND	87	81
16	pg/mL	ND	ND	ND	47.97	78.96
	% Expected value	ND	ND	ND	87	80

ND – Not Detected – below product dynamic range

Native CYR61 was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

Dilution Factor	Interpolated value	25% HeLa Cell Culture Supernatant	25% MDA-MB-453S Cell Cutlure Supernatant
Undiluted	pg/mL	2,199	572.5
	% Expected value	100	100
2	pg/mL	1,306	254.1
	% Expected value	119	89
4	pg/mL	514.6	118.6
	% Expected value	94	83
8	pg/mL	226.4	57.96
	% Expected value	82	81
16	pg/mL	110.3	28.80
	% Expected value	80	80

Native CYR61 was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in 1X Cell Extraction Buffer PTR.

Dilution Factor	Interpolated value	250 µg/mL HeLa Cell Extract	500 µg/mL MDA-MB-453S Cell Extract
Undiluted	pg/mL	1,059	1,784
	% Expected value	100	100
2	pg/mL	518.7	1,054
	% Expected value	98	118
4	pg/mL	268.5	427.6
	% Expected value	101	96
8	pg/mL	138.9	187.3
	% Expected value	105	84
16	pg/mL	75.06	92.64
	% Expected value	113	83

Precision

Mean coefficient of variations of interpolated values of CYR61 from three concentrations of HeLa cell culture supernatant within the working range of the assay.

	Intra-assay	Inter-assay
N=	5	3
CV (%)	2.5	3.8

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
https://www.abcam.com/en-us/technical-resources/guides/elisa-guide

Technical Support

Version 1a | 2026-04-27

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