ab325456 – Human LRG SimpleStep ELISA® Kit (LRG1), Chemiluminescent

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

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, Individual Serum Donors, and Rheumatoid Arthritis sample data were collected using the colorimetric version of this kit (ab260066).

Materials Supplied

Item	Quantity 1 x 96 tests	Quantity 10 x 96 tests	Storage Condition
Human LRG Capture Antibody 10X	600 µL	10 x 600 µL	+4°C
Human LRG Detector Antibody 10X	600 µL	10 x 600 µL	+4°C
Human LRG Lyophilized Recombinant Protein	2 Vials	10 x 2 Vials	+4°C
Antibody Diluent CPI2	6 mL	10 x 6 mL	+4°C
Sample Diluent NS	50 mL	2 x 250 mL	+4°C
Wash Buffer PT 10X	20 mL	200 mL	+4°C
ChemiHRP Reagent A	3 mL	50 mL	+4°C
ChemiHRP Reagent B	3 mL	50 mL	+4°C
SimpleStep Pre-Coated Black 96-Well Microplate	96 Wells	10 x 96 Wells	+4°C
Plate Seal	1	10	+4°C

Materials Required, Not Supplied

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

Luminometer with the following settings: 0.5-1 second/well read time; summation mode (all wavelengths).
Deionized water.
Multi- and single-channel pipettes.
Tubes for standard dilution.
Plate shaker for all incubation steps.
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.

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.

Additional Wash Buffer Availability

Customers who wish to increase wash stringency through up to 6 total wash cycles, longer wash soak times, higher wash volumes, or the use of automated plate washing systems may purchase 10X Wash Buffer PT (ab206977) separately. This wash buffer can be used to prepare larger volumes of wash solution and is particularly beneficial for laboratories performing high-throughput testing or implementing automated washing workflows.

Use of bulk wash buffer may also be advantageous when evaluating assay optimization strategies, including wash cycle counts up to 6 total washes or extended wash incubations designed to further reduce background signal and improve assay sensitivity.

Antibody Cocktail: Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent CPI2. 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 CPI2. 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).

1. Reconstitute the LRG 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 60,000 pg/mL **Stock Standard** Solution.
2. Label nine tubes, Standards 1– 9.
3. Add 150 µL of Sample Diluent NS into tube numbers 1-9.
4. Use the **Stock Standard** to prepare the following dilution series. Standard #9 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**	75	150	60,000	20,000
2	Standard#1	75	150	20,000	6,666.7
3	Standard#2	75	150	6,666.7	2,222.2
4	Standard#3	75	150	2,222.2	740.7
5	Standard#4	75	150	740.7	246.9
6	Standard#5	75	150	246.9	82.3
7	Standard#6	75	150	82.3	27.4
8	Standard#7	75	150	27.4	9.1
9	Blank Control	0	150	0	0

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	**Range**
Serum	1:320,000 – 1:10,000
Plasma – Citrate	1:160,000 – 1:5,000
U937 Cell Culture Supernatant	0.78 – 25%

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. Dilute samples at least 1:10,000 into Sample Diluent NS and assay. Store un-diluted serum at -20°C or below. Avoid repeated freeze-thaw cycles.

Plasma Collect plasma using citrate. Centrifuge samples at 2,000 x g for 10 minutes. Dilute samples at least 1:5,000 into Sample Diluent NS and assay. Store un-diluted plasma samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

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

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 4°C.

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 4°C 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 3 times with 350 µL 1X Wash Buffer PT per wash. Completely aspirate or decant the contents of each well before each wash, then immediately dispense 350 µL 1X Wash Buffer PT into each well. Allow the wash buffer to remain in the wells for at least 30 seconds during each wash cycle. After the final wash, carefully invert the plate and tap firmly on clean absorbent paper towels to remove residual liquid. Complete removal of wash buffer is essential to minimize background and maximize assay performance.

   Best Practice Recommendations: For applications requiring the lowest limits of detection or highest precision, consider the following optimization strategies:
   - Increase the number of wash cycles beyond the standard protocol, up to a maximum of 6 total wash cycles.
   - Extend wash soak times to 60 to 120 seconds.
   - Verify complete aspiration during each wash cycle.
   - Ensure automated plate washers are properly calibrated and aligned.
   - Use sufficient wash volume to completely fill each well during every wash step.

   These modifications can help reduce nonspecific background, improve signal-to-noise ratio, enhance low-end sensitivity, and improve replicate precision.
   Important: Complete well filling, adequate soak time, thorough aspiration, and effective residual liquid removal are among the most important factors influencing overall assay performance and reproducibility.
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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Additional information

ASSAY SPECIFICITY

This kit is designed for the quantification of human LRG.

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

Native signal was detected in serum, plasma (citrate), and cell culture supernatant.

Plasma (heparin), plasma (EDTA), saliva, urine, milk, CSF, cell extract, tissue extract samples have not been tested with this kit.

SPECIES REACTIVITY

Other species reactivity was determined by measuring 1:40,000 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.

Reactivity < 3% was determined for the following species: Mouse, Rat, Cow

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	3,225	3,225	3,225
9.1	6,481	6,289	6,385
27.4	12,904	12,368	12,636
82.3	32,630	31,754	32,192
246.9	94,797	91,226	93,012
740.7	285,660	275,680	280,670
2,222.2	834,810	810,150	822,480
6,666.7	2,522,500	2,380,900	2,451,700
20,000	6,745,000	6,745,000	6,745,000

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

TYPICAL SAMPLE VALUES

Sensitivity:

The calculated minimal detectable dose (MDD) is 3.2 pg/mL. The MDD was determined by calculating the mean of zero standard replicates (n=8) and adding 2 standard deviations then extrapolating the corresponding concentration.

Recovery

LRG recombinant standard was spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery
Serum (1: 40,000)	92
Plasma – Citrate (1: 40,000)	95
U937 Cell Culture Supernatant (6.25%)	86

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.

Native LRG 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	1: 10,000 Human Serum	1: 5,000 Human Plasma (Citrate)	25% U937 Supernatant
Undiluted	pg/mL	1,778	2,093	1,717
	% Expected value	100	100	100
2	pg/mL	855	1,048	871
	% Expected value	96	100	101
4	pg/mL	438	521	431
	% Expected value	98	100	100
8	pg/mL	213	248	206
	% Expected value	96	95	96
16	pg/mL	116	124	103
	% Expected value	104	95	96

Precision

Mean coefficient of variations of interpolated values of LRG from a single concentration of serum within the working range of the assay.

	Intra-assay
N=	8
CV (%)	5.4

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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