Seravue® LC-SPIK ELISA cGMP Kit

Kit ID # LCS-001 cGMP

96 assays

Manual Version: 01

Investigational Use Only (IUO)

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For Research Use Only. Not for Use in Diagnostic Procedures.

1. Intended Use for Research: The Seravue® LC-SPIK ELISA Kit is designed exclusively for research applications to be performed by trained laboratory professionals. Its purpose is the quantitative determination of LC-SPIK levels in human serum samples within a controlled research setting. The performance characteristics of this product for any diagnostic or clinical use have not been established. Data generated should not be used to inform patient care, be added to a medical record, or be relied upon for any clinical decision-making.

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Product description

The Seravue® LC-SPIK ELISA Kit is an enzyme-linked immunosorbent assay for quantitative detection of human LC-SPIK in serum samples.

Background

Liver cancer, primarily in the form of hepatocellular carcinoma (HCC), is the second most lethal cancer worldwide. HCC accounts for 90% of liver cancer cases and is closely linked to risk factors such as chronic viral infections (HBV/HCV), fatty liver, cirrhosis, alcohol abuse, and metabolic diseases like diabetes. In 2020, HCC led to 906,000 new cases and 830,000 deaths globally, highlighting the urgent need for effective surveillance and early detection to improve patient survival. [1-4]

Serum protein biomarkers play a crucial role in the diagnosis and surveillance of HCC. Alpha-fetoprotein (AFP) is the most commonly used marker, but its limited sensitivity, especially in early-stage HCC, and potential for false positives make it less reliable as a standalone diagnostic tool.[5,6] Other biomarkers like AFP-L3 and des-gamma-carboxyprothrombin (DCP) show some enhancing sensitivity and specificity,[7-9] however, they are still needed to improve, particularly in the detection of early stage HCC. Early diagnosis is critical for improving survival, and serum biomarkers are essential for effective surveillance and timely intervention in HCC.

Liver cancer–secreted serine protease inhibitor Kazal (LC-SPIK) is a protein that is specifically elevated in cases of hepatocellular carcinoma (HCC). LC-SPIK is a liver cancer–specific isoform of serine protease inhibitor Kazal (SPIK), which also called pancreas secretory trypsin inhibitor and tumor-associated trypsin inhibitor. [10,11] Unlike the typical SPIK, which is predominantly found in pancreatic tissue and can be elevated in conditions such as acute pancreatitis, [12] LC-SPIK is uniquely secreted by liver cancer cells. [13,14] This specificity makes LC-SPIK a highly selective biomarker for liver cancer. Our clinical studies show LC-SPIK performance in detection of HCC, particular in its early stages, is significantly better than AFP. Furthermore, LC-SPIK accurately detected the presence of HCC in more than 70% of HCC patients with false-negative AFP test results. [15] The LC-SPIK ELISA kit Seravue® is specifically designed to detect LC-SPIK from human serum.

Principle of the Assay

Seravue® is a quantitative ELISA that measures the concentration of LC-SPIK in human serum to aid determination of HCC in patients. LC-SPIK is specific to HCC and distinguished from SPIK by the retention of an additional N-terminal fragment during protein secretion. Test has negligible affinity to SPIK protein, a protein formed in the pancreas and will not detect or be influenced by presence of SPIK in serum.

The Seravue® ELISA kit uses a 96-well strip well microplate pre-coated with a capture antibody that specifically binds to LC-SPIK, the target analyte (1). When experimental samples, such as patient serum or standards, are added to the wells, the LC-SPIK in the sample binds to the immobilized capture antibody selectively (2). A horseradish peroxidase (HRP)-conjugated detection antibody that binds to a different epitope on the LC-SPIK facilitates detection when added to the assay wells. Tetramethylbenzidine (TMB) substrate solution is then added to the wells, where it reacts with the HRP enzyme, producing a blue color to the assay wells. The intensity of the blue color is proportional to the amount of LC-SPIK in the sample (3). The reaction is stopped by the addition of an acidic stop solution, turning the color from blue to yellow. The absorbance of the resulting yellow color is measured at 450 nm, and this absorbance is directly proportional to the concentration of LC-SPIK in the sample. The assay performance is validated using positive (contains native human LC-SPIK) and negative internal controls. Figure 1 demonstrates the assay principle of the sandwich ELISA in an assay well of a 96-well plate. The concentration of LC-SPIK in each sample is determined by comparing the absorbance values to a 4-parameter logistic (4PL) standard curve constructed from known calibrator concentration.

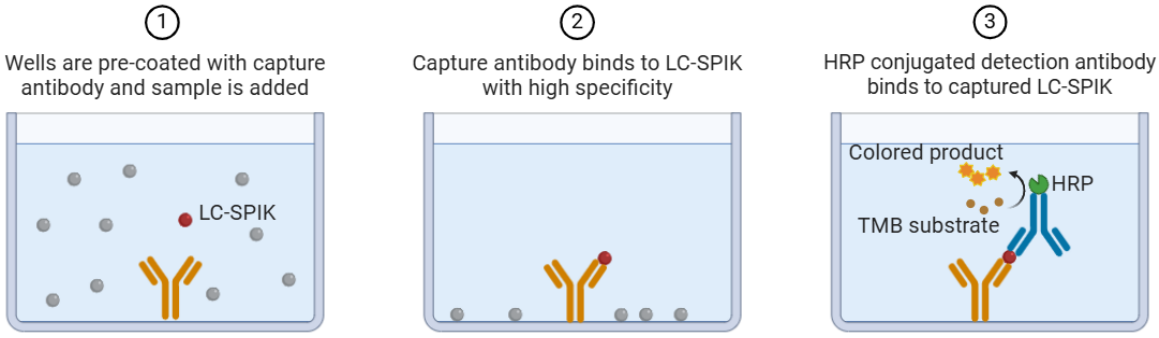


Figure 1: Schematic representation of Seravue ELISA assay principle demonstrating capture antibody coated at the bottom of the ELISA well. LC-SPIK present in the samples were captured by capture antibody while all the non-specific proteins are washed away. The captured LC-SPIK is detected by addition of HRP conjugated capture antibody where TMB substrate results in color read-out measured by absorbance reader at 450 nm.

Contents & Storage

Unopened test kits should be stored at 2-8°C upon receipt. The unopened kit may be stored at 2-8°C for up to 12 months. The shelf-life of the kit is shortened after opening the kit. The microtiter plate should be kept in a sealed bag with desiccants.

Component	Amount
96-strip well microplate – 12 modules of 8 wells each *Supplied in a foil pouch. Ready to use.*	96 (8x12) wells
Calibrators A-G (10.0, 5.00, 2.50, 1.25, 0.62, 0.31, 0.0 ng/mL) *Supplied at 10X concentration in a foil pouch. Ready to use.*	7 vials, 100 µL each
Positive control – cellular media containing native human LC-SPIK Negative control – cellular media without LC-SPIK *Supplied at 10X concentration in a foil pouch. Ready to use.*	2 vials, 100 µL each
Dilution Buffer contains PBS, BSA *Ready to use.*	25 mL
Detection antibody - Enzyme conjugate, HRP labeled *Supplied at 100X concentration. Ready to use.*	200 µL
TMB Solution *Ready to use.*	11 mL
Stop solution, contains acid *Ready to use.*	11 mL
Wash Buffer - contains detergent *Supplied at 20x concentration. Ready to use.*	2 bottles, 50 mL

Materials required but not supplied

- Microplate reader capable of endpoint measurements at 450 nm
- 10 µL to 1000 µL adjustable single/multi-channel micropipettes with disposable tips
- Plate washer – optional
- Distilled or deionized water for dilutions
- Mixer for plate shaking
- Data reduction software for 4PL curve fit (available at imcarebiotech.com)

Reagent Preparation

- All reagents should be allowed to reach room temperature (18-25 °C) before use.
- All reagents are provided with 2 mL tubes, we recommend spinning the tubes prior to use.
- **Standard and Samples:** Standard is set at a 10x concentrate for each standard level (10, 5, 2.5, 1.25, 0.625, 0.31 ng/mL). The standards, samples, and positive control will be diluted 10x in the plate by first adding 90 µL dilution buffer to the plate followed by 10 µL standard/control/sample.
- **Wash Buffer:** Dilute the concentrated wash buffer 20-fold with distilled water (add 50 mL concentrated wash buffer into 950 mL distilled water). Prime plate washer with wash buffer.
- **HRP Labeled Detection Antibody Solution:** Prepare no more than 1 hour before experiment. Calculate the total volume of working solution required. (100 µL x number of wells x 1.1). Dilute 100x detection antibody 100-fold with dilution buffer and mix thoroughly (do not vortex).

Assay Procedure

Equilibrate all kit components and samples to room temperature prior to use. The approximate assay time is 2 hour 15 minutes.

1. Secure the desired number of coated wells in the holder.

2. **Prepare Plate & Sample addition:** Set standard, test sample, and control wells on the pre-coated plate and record their positions. Add 90 µL dilution buffer solution to the bottom of all wells without scraping the side walls.
    a. Add 10 µL of the diluted standards into the standard wells.
    b. Add 10 µL 0 ng/mL calibrator and dilution buffer to blank wells.
    c. Add 10 µL positive control into control wells.
    d. Add 10 µL of human serum samples into test sample wells.
    e. Place plate on plate mixer and mix for 30 seconds at 1000 RPM. It is very important to have complete mixing in this step.

3. **Sample Incubation:** Cover the plate and incubate at 37°C for 60 minutes.

4. **Wash:** Remove cover and wash plate 5 times with wash buffer and dry by flipping the plate and tapping on a dry paper towel.

5. **Detection Antibody:** Add 100 µL of 1X HRP labeled detection antibody solution to all wells without touching the sides of the wells.

6. **Detection Antibody Incubation:** Cover plate and incubate at 37°C for 45 minutes.

7. **Wash:** Remove cover and wash plate 5 times with wash buffer and dry by flipping the plate and tapping on a dry paper towel.

8. **Substrate:** Add 100 µL of TMB substrate to all the wells.

9. **Substrate Incubation:** Cover the plate and incubate at room temperature in dark for 20 minutes.

10. **Stop Solution:** Add 100 µL stop solution to all the wells.

11. **Measurement:** Read OD absorbance at 450 nm in microplate reader immediately after adding stop solution.

Calculation of Results

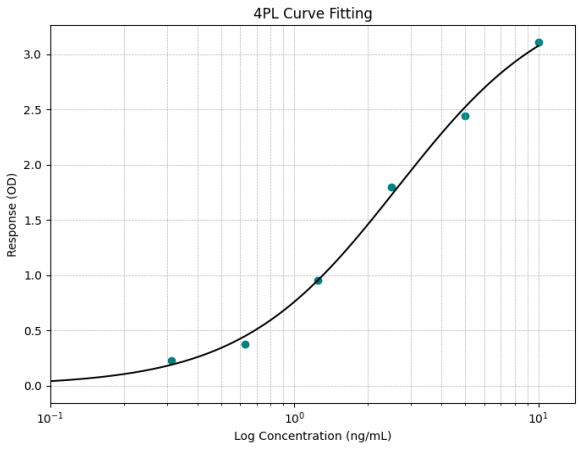
- Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20 percent of the mean value.

- Construct a standard curve by plotting the mean absorbance obtained for each standard calibrator against its concentration in ng/ml with absorbance on y-axis and concentration on x-axis.
- Drawing a best fit curve through the points of the graph, we recommend a 4PL Curve as best curve fit to calculate sample concentrations using mean absorbance value for each sample.
- If instructions in this protocol have been followed, samples have been diluted 1:10 (10 µL sample + 90 µL dilution buffer). Thus, concentrations read from the standard curve must be multiplied by the dilution factor of x10.
- Calculation of samples with a concentration exceeding 100 ng/mL may result in incorrect LC-SPIK levels. Such samples require further dilution according to expected human LC-SPIK values with dilution buffer in order to have accurate concentration evaluation.

Example of Standard Curve

Results of a typical standard run with OD readings at 450 nm shown on the Y-axis against LC-SPIK standard concentrations shown on the X-axis. This standard curve is for the purpose of illustration only and should not be used to calculate unknowns. Each user should obtain their own data and standard curve.

LC-SPIK (ng/mL)	OD at 450 nm
10.0	3.12
5.00	2.45
2.50	1.81
1.25	0.96
0.62	0.38
0.31	0.23
0.00	0.01



4PL Fitting Properties	Value
Top	3.59
Bottom	0.00
IC50	2.64
logIC50	0.42
Hill Slop	1.35
Span	3.59
RSQ	1.00
LOD	0.00

Figure 2: Representative standard curve for LC-SPIK Seravue® ELISA kit.

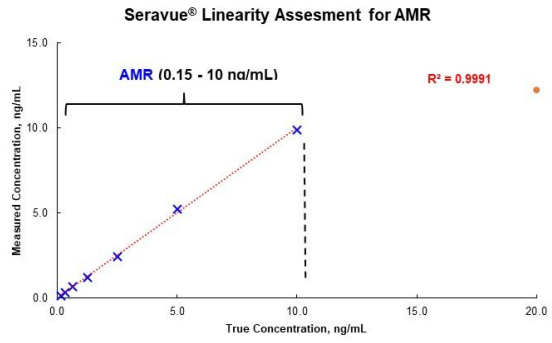
Best Practices

- Wear appropriate Personal Protective Equipment (PPE) when handing the samples and other kit components.
- A new standard curve must be created for each assay run, as conditions can vary from one assay to another for accurate sample analysis.
- Proper washing at each step is crucial. Inadequate or improper washing can result in false positive or false negative results. Ensure the assay wells are empty prior to the addition of follow-up reagents.
- Ensure wells are not left uncovered or allowed to dry for long periods.
- Bacterial or fungal contamination in either the screen samples or reagents, or cross-contamination between reagents, can lead to inaccurate results.
- Use disposable pipette tips, flasks, and glassware when possible. If reusable glassware is used, ensure it is thoroughly cleaned and rinsed to remove all detergent residues.

Performance Characteristics

Sensitivity: The limit of detection (LoD) of LC-SPIK is defined as the lowest concentration of analyte in a sample that can be distinguished from background noise with high confidence (Mean of blank + 3x standard deviation). LoD of the Seravue LC-SPIK ELISA kit was determined to be ~ 0.043 ng/mL. The limit of quantitation (LoQ) defined as Mean of blank + 10x standard deviation was determined to be ~ 0.055 ng/mL.

Analytical Measurement Range (AMR): AMR of the Seravue LC-SPIK ELISA kit defines the range over which the assay provides precise measurements of the analyte. The AMR was established by evaluating the linearity and recovery of known analyte concentrations across multiple assay runs. Measured concentrations were compared to expected values, and %recovery was assessed against an acceptance criterion of 80%–120%.AMR of the Seravue LC-SPIK ELISA kit was determined as 0.15 to 10.00 ng/mL, demonstrating high correlation (R^2 = 0.999) with true values ensuring reliable quantitation of the LC-SPIK.



True Value (ng/mL)	Measured Value (ng/mL)	% recovery
20.00	12.20	61.00
10.00	9.90	98.97
5.00	5.26	104.32
2.50	2.42	96.72
1.25	1.22	98.00
0.62	0.67	107.04
0.31	0.34	109.44
0.15	0.14	90.88

Figure 3: Representative linearity assessment curve for LC-SPIK Seravue® ELISA kit.

Repeatability:

Five individual assays were performed to assess variance and %CV values, establishing the repeatability of the test. In each run, three independent measurements were taken for each test sample, which included two test samples and negative control (n=15 measurements). Test sample 1 consisted of human serum with approximately 20 ng/mL of LC-SPIK, and test sample 2 contained human serum with approximately 70 ng/mL of LC-SPIK, while the negative control contained human serum with approximately 1.0 ng/mL of LC-SPIK. 2 standard curves were run on each plate. Internal quality control (QC) levels - HQC, MQC, and LQC - were included in each run to monitor assay performance, demonstrating inter-assay CV% values < 11%.

Variance Limits (±20% Tolerance Range)

Level	Observed Variance (S^2)	Acceptable Range for S^2
Level 1	0.0705	0.0565 – 0.0846
Level 2	0.4623	0.3698 – 0.5547

Coefficient of Variation (CV%) Limits (±20% Tolerance Range)

Level	Observed CV %	Acceptable Range for CV
Level 1	10.64	8.51 %– 12.76 %
Level 2	9.61	7.68 % – 11.53 %

Interference

No interference has been observed with hemolytic samples (up to 5 mg/dL hemoglobin), lipemic samples (up to 10 g/dL triglycerides), or samples containing bilirubin (up to 20 mg/dL) in either serum or plasma. Hemolyzed samples with hemoglobin concentration > 5 mg/dL should be avoided due to potential interference observed. Additionally, no interference has been noted with the use of common anticoagulants (Citrate, EDTA, Heparin). However, for optimal results, it is recommended to avoid using grossly hemolyzed or lipemic samples

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