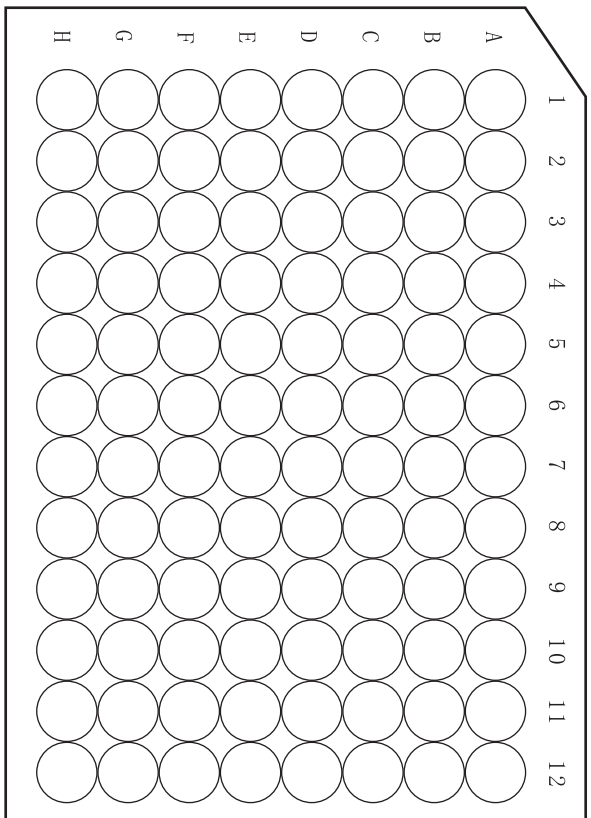

Human CD19 Immunoassay

Catalog Number:SEKH-0121

For the quantitative determination of human CD19 concentrations in cell culture supernates, serum, and plasma.

For research use only. Not for use in diagnostic procedures.

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LINEARITY: To assess the linearity of the assay, three samples were spiked with high concentrations of CD19 in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay. (The plasma samples were initially diluted 1:1)

The linearity of the assay

Dilution ratio	Recovery(%)	Citrate plasma	Cell culture supernatants
1:2	Average% of Expected	95	97
	Range(%)	87-106	90-111
1:4	Average% of Expected	93	103
	Range(%)	85-108	94-117

Performance Characteristics

SENSITIVITY: The minimum detectable dose was 7.8pg/mL.
SPECIFICITY: This assay recognizes both natural and recombinant human CD19. The factors listed below were prepared at 100ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

REPEATABILITY: The coefficient of variation of both intra-assay and inter-assay were less than 10%.
RECOVERY: The recovery of CD19spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Recovery of CD19 in two matrices

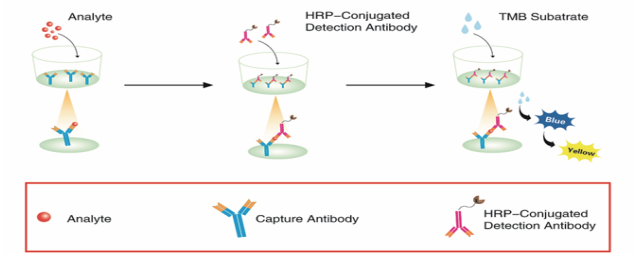
Sample Type	Average % of Expected Range(%)	Range(%)
Citrate plasma	92	84-105
Cell culture supernatants	94	83-107

BACKGROUND

The cluster of differentiation (CD) system is commonly used as cell markers in Immunophenotyping. Different kinds of cells in the immune system can be identified through the surface CD molecules associating with the immune function of the cell. There are more than 320 CD unique clusters and subclusters have been identified. Some of the CD molecules serve as receptors or ligands important to the cell through initiating a signal cascade which then alter the behavior of the cell. Some CD proteins do not take part in cell signal process but have other functions such as cell adhesion. Cluster of differentiation 19 (CD19) is a member of CD system. CD19 is a cell surface molecule that assembles with the antigen receptor of B-cells. This results in a descent in the threshold for antigen receptor-dependent stimulation. A simplified view holds that the ability of B-cells to respond to the various antigens in a specific and sensitive manner is achieved in the presence of low-affinity antigen receptors. CD19 primarily acts as a B-cell co-receptor in conjunction with CD21 and CD81. The formation of the receptor complex is induced by antigen and CD19, induced by exogenous antigen, has been found cytoplasmic tail phosphorylated and bind to sIg.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for CD19 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any CD19 present is captured by the coated antibody after incubation. Following extensive washing, a HRP-conjugated antibody specific for CD19 is added to detect the captured CD19 protein in sample. The wells are then washed to remove unbound HRP-conjugated antibody and Tetramethyl-benzidine (TMB) reagent is added. Incubated at room temperature, only those wells that contain CD19, HRP-labled antibody will appear blue in color. Color development is stopped by the addition of Stop Solution, changing the color to yellow, and optical density is measured spectrophotometrically at 450nm. The OD value is proportional to the concentration of Human CD19.



TECHNICAL HINTS AND LIMITATIONS

1.This Solarbio ELISA should not be used beyond the expiration data on the kit label.
2.To avoid cross-contamination, use a fresh reagent reservoir and pipette tips for each step.
3.To ensure accurate results, some details, such as technique, plasticware and water sources should be emphasized.
4.A thorough and consistent wash technique is essential for proper assay performance.
5.A standard curve should be generated for each set of samples assayed.
6.It is recommended that all standards and samples be assayed in duplicate.
7.Avoid microbial contamination of reagents and buffers. Buffers containing protein should be made under aseptic conditions and be prepared fresh daily.
8.In order to ensure the accuracy of the results, the standard curve should be made every time.

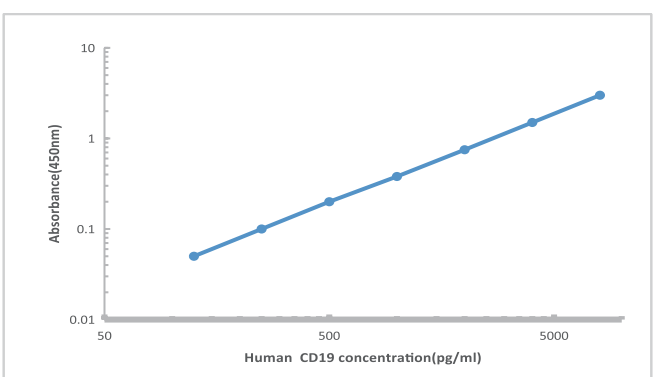
PRECAUTIONS

The Stop Solution suggested for use with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
5.This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Typical data using the CD19 ELISA

Standared(ng/ml)	OD.	OD.	Average	Corrected
0	0.025	0.018	0.021	-
125	0.191	0.182	0.187	0.165
250	0.278	0.265	0.271	0.250
500	0.413	0.394	0.403	0.382
1000	0.656	0.626	0.641	0.619
2000	1.066	1.016	1.041	1.019
4000	1.735	1.654	1.695	1.673
8000	2.827	2.696	2.761	2.740



Representative standard curve for CD19 ELISA.

ASSAY PROCEDURE

Prepare all reagents and standards as directed,wash the plate 3 times before the assay.

⇩

Add 100μl standard or samples to each well,
and incubate 120 minutes at room temperature(25±2℃).

⇩ Aspirate and wash 4 times

Add 100μl working solution of HRP-Conjugate anti- human CD19 antibody to each well, and incubate 60 minutes at room temperature(25±2℃).

⇩ Aspirate and wash 4 times

Add 100μl Substrate solution to each well, incubate 5-20 minutes (depending on signal) at room temperature(25±2℃).Protect from light.

⇩

Add 50μl Stop solution to each well. Read at 450nm within 5 minutes.

CALCULATION OF RESULTS

1. The standard curve is used to determine the amount of specimens.
2. First, average the duplicate readings for each standard, control, and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.
3. Construct a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.
4. The data may be linearized by plotting the log of the CD19 concentrations versus the log of the O.D. and the best fit line can be determined by

KIT COMPONENTS& STORAGE CONDITIONS

PART	SIZE	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Microwell Plate - antibody coated 96-well Microplate (8 wells ×12 strips)	1 plate	Return unused wells to the foil pouch containing the desiccant pack.Reseal along entire edge of the zip-seal.May be stored for up to 1 month at 2 – 8℃**
Standard - lyophilized, 8000pg/ml upon reconstitution	2 vials	Store at 2-8°C **for six months
Concentrated HRP-Conjugated antibody(100X) - 120 ul/vial	1 vial	Store at 2-8°C **for six months
Standard /sample Diluent - 16 ml/vial	1 bottle	Store at 2-8°C **for six months
HRP-Conjugated Diluent - 16 ml/vial	1 bottle	Store at 2-8°C **for six months
Wash Buffer Concentrate (20x) - 30 ml/vial	1 bottle	Store at 2-8°C **for six months
Substrate Solution - 12 ml/vial	1 bottle	Store at 2-8°C **for six months
Stop Solution - 12 ml/vial	1 bottle	Store at 2-8°C **for six months
Plate Cover Seals	4 pieces	Store at 2-8°C **for six months

**Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED

1.Microplate reader capable of measuring absorbance at 450 nm.
2.Pipettes and pipette tips.
3.Deionized or distilled water.
4.Squirt bottle, manifold dispenser, or automated microplate washer.
5.500 mL graduated cylinder.

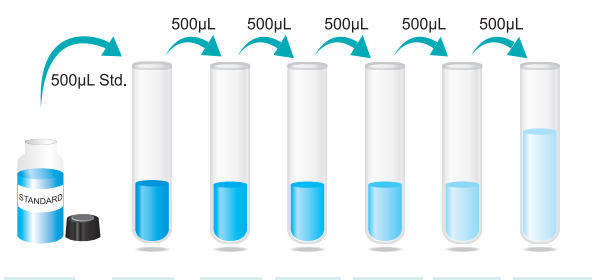
SPECIMEN COLLECTION & STORAGE

Cell Culture Supernates - Centrifuge cell culture media at 1000g (or 3000rpm) to remove debris. Assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.
Serum - Use a serum separator tube (SST) and allow samples to clot for 2 hours at room temperature or overnight at 2-8℃.Centrifuge approximately for 15 minutes at 1000g(or 3000rpm). Assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.
Plasma -Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000g(or 3000rpm) within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.
Note: It is recommended to conduct a pre-test before the formal experiment to determine the dilution ratio.

REAGENTS PREPARATION

1. **Temperature returning** - Bring all kit components and specimen to room temperature(20-25℃) before use.
2. **Wash Buffer** - Dilute 30mL of Wash Buffer Concentrate with 570mL of deionized or distilled water to prepare 600mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

3. **Standard** - Reconstitute the Standard with 1mL of Standard/Sample Diluent. This reconstitution produces a stock solution of 8000pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 500μL of Standard/Sample Diluent into 4000pg/ml tube and the remaining tubes. Use the stock solution of 8000pg/mL to produce a 2-fold dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 8000pg/mL standard serves as the high standard. TheStandard/Sample Diluent serves as the zero standard (0 pg/mL).
   ***If you do not run out of re-melting standard, store it at -20℃. Diluted standard shall not be reused.**
4. **Working solution of HRP-**Congugated Antibody(100×):Make a 1:100 dilution in Reagent Diluent. If the entire 96-well plate is used, add 100uL of HRP Conjugate to 10mL of HRP-Congugated Diluent to make working dilution of HRP Conjugate and mix thoroughly prior to the assay. The rest of undiluted HRP Conjugate can be stored at 4°C for up to 6 months.
   DO NOT FREEZE
   ***The working solution should be used within one day after dilution.**



Preparation of CD19 standard dilutions