INTENDED USE

The Camrelizumab Anti-PD-1 ELISA Kit is an indirect ELISA for quantifying active (PD-1-binding) Camrelizumab in serum/plasma and drug processing and/or other biological solutions. This test is designed for human samples but can be used for other species (e.g., mouse, rabbit, monkey) after proper validations. For research use only (RUO); not for diagnosis, cure, or prevention of the disease.

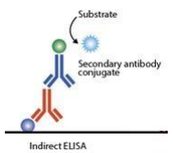
GENERAL INFORMATION

Camrelizumab is a human IgG4/kappa monoclonal antibody produced by recombinant DNA technology and designed to block the action of Programmed Cell Death Protein 1 (**PD-1**), a major surface protein component of the **PD-1/PD-L1** T cell immune checkpoint.

Immune checkpoints are regulators of immune activation which play key roles in maintaining immune homeostasis and preventing autoimmunity. Programmed Cell Death Protein 1 (PD-1) is primarily expressed on mature T cells in peripheral tissues and the tumor microenvironment. PD-L1, found on some normal cells and in higher-than-normal amounts on some types of cancer cells, represent a checkpoint involved in the suppression of the immune system and occurs following infection to limit the killing of bystander host cells and prevent autoimmune disease. When PD-L1 binds to PD-1, it keeps T cells from killing the PD-L1-containing cells, including the cancer cells. This action then is an escape mechanism for PD-L1 producing tumor cells to avoid T cell-mediated death.

Camrelizumab, indicated as a drug for treatment of a wide range of cancers, inhibits the PD-1/PD-L1 checkpoint by binding to PD-L1, which then alleviates the suppression of T cells and thereby allows their killing of tumor cells in the microenvironment.

PRINCIPLE OF THE TEST



The Camrelizumab ELISA kit is based upon capture of active Camrelizumab to PD-1 antigen coated on the plate. Bound Camrelizumab is then detected by anti-Human IgG HRP conjugate. After a washing step, chromogenic substrate (TMB) is added and color (blue) develops, which is directly proportional to the amount of antibody present in the sample. Stop Solution is added (converts blue to yellow color), and A450nm is then measured using an ELISA reader. The activity of antibody in samples is calculated relative to supplied active standards.

KIT CONTENTS

The microtiter well plate and all other reagents, if unopened, are stable at 2-8°C until the expiration date printed on the box label. Stabilities of the working solutions are indicated under Reagent Preparation.

To Be Reconstituted: Store as indicated.

Component	Preparation Instructions
Sample Diluent Concentrate (20x) Cat.# SD-20T, 10ml	Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as **Working Sample Diluent** and store at 2-8°C until the kit lot expires or is used up.
Wash Solution Concentrate (100x) Cat.# WB-100, 10ml	Dilute the entire volume 10ml + 990ml with distilled or deionized water into a clean stock bottle. Label as **Working Wash Solution** and store at ambient temperature until kit is used entirely.
Anti-Human IgG-HRP Conjugate Concentrate (100x) Part No. H-HuG4-612, 0.15ml	In buffer with protein, detergents, and antimicrobial. Dilute fresh as needed; 10 ul of concentrate to 1 ml of **Working Sample Diluent** (**WSD**) is sufficient for 1 8-well strip. Use within the working day and discard. Return 100X to 2-8°C storage.

Ready For Use: Store as indicated on labels.

Component	Part	Amt	Contents
PD-1 Antigen Coated Strip Plate	200-561	8-well strips (12)	Coated with PD-1 antigen and post-coated with stabilizers.
Camrelizumab Standards			
2.5 ng/ml 5 ng/ml 10 ng/ml 20 ng/ml 40 ng/ml	200-587B 200-587C 200-587D 200-587E 200-587F	0.65 ml 0.65 ml 0.65 ml 0.65 ml 0.65 ml	Five (5) vials, each containing purified recombinant Camrelizumab of designated concentrations; diluted in buffer with protein, detergents and anti-microbial.
Positive Control [range on label]	200-587PC	0.65 ml	Camrelizumab of stated concentration range; diluted in buffer with protein, detergents and anti-microbial.
TMB Substrate	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	Dilute sulfuric acid.

Materials Required But Not Provided:

- Pipettors and pipettes that deliver 100ul and 1-10ml. A multi-channel pipettor is recommended.
- Disposable glass or plastic 5-15ml tubes for diluting samples and Antibody HRP Concentrate.
- Graduated cylinder to dilute Wash Concentrate; 0.2 to 1L.
- Stock bottle to store diluted Wash Solution; 200ml to 1L.
- Distilled or deionized water to dilute reagent concentrates.
- Microwell plate reader at 450 nm wavelength.

ASSAY DESIGN AND SET-UP

Sample Collection and Handling

Culture medium, serum, and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference (See Limits of the Assay, page 6). For **serum**, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature. For all samples, clarify by centrifugation and/or filtration. If samples will not be assayed immediately, store frozen for long-term storage.

DILUTE serum samples in Working Sample Diluent. Dilutions of 1:50–1:100 may be appropriate for standard drug treatment regimens or for drug production processing.

Prepare additional dilutions as required to bring expected Camrelizumab concentrations within the range of the Standard curve. Samples reading above the top standard should be diluted further and re-tested. Diluted samples are stable for several months refrigerated, and longer if stored frozen.

Assay Validation

Validate the performance of the Camrelizumab sample and matrix in the assay system for recovery (see Limits of the Assay, page 6), as follows:

Recovery – a measure of the interference of the sample matrix in providing accurate quantitation of Camrelizumab in the sample relative to the Camrelizumab Standards.

Prepare and run a series of dilutions of the Camrelizumab sample (within the Standard range) in Working Sample Diluent to determine the dilutions that give consistent and accurate quantitation. Non-human serum and plasma may require 1:50 dilution or greater to obtain consistent quantitation or complete antigen recovery. Full Recovery from processing solutions will likely require much less dilution.

Plate Set-up

Bring all reagents to room temperature (18-30°C) equilibration (at least 30 minutes).

- Determine the number of wells for the assay run. Duplicates are recommended, including 10 Standard wells and 2 wells for each sample and control to be assayed.
- Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.
- Add 200-300ul Working Wash Solution to each well and let stand for about 1-5 minutes. Aspirate or dump the liquid and pat dry on a paper towel before sample addition.

Assay Procedure

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

1. 1st Incubation [100ul – 60 min; 4 washes]

- Add 100ul of sample diluent (blank), calibrators, samples, and controls each to pre-determined wells.
- Tap the plate gently to mix reagents and incubate for **60 minutes.**
- Wash wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer may be used. Improper washes may lead to falsely elevated signals and poor reproducibility.

2. 2nd Incubation [100ul – 30 min; 5 washes]

- Add 100ul of diluted **Anti-Human IgG4-HRP Conjugate** to each well.
- Incubate for 30 minutes.
- Wash wells 5 times as in step 1.

3. Substrate Incubation [100ul – 15 min]

- Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.
- Incubate for 15 minutes in the dark, e.g., place in a drawer or closet.

Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, or read OD at 405-410 nm (results are valid).

4. Stop Step [Stop: 100ul]

- Add 100ul of Stop Solution to each well.
- Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

5. Absorbance Reading

- Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings, and data calculations if available.
- Read absorbance of the entire plate at 450nm using a single wavelength within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.

PRECAUTIONS AND SAFETY INSTRUCTIONS

Calibrators, Sample Diluent, and Antibody HRP contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains dilute sulfuric acid. Follow good laboratory practices and avoid ingestion or contact of any reagent with skin, eyes, or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water. MSDS for TMB, sulfuric acid, and BND can be requested or obtained from the ADI website.

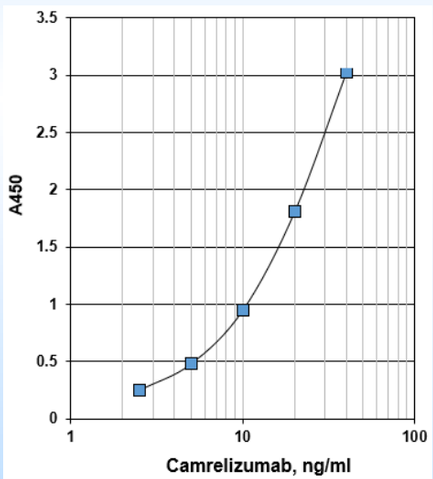
CALCULATION OF RESULTS

1. The results may be calculated using any immunoassay software package. The four-parameter curve-fit is recommended. If software is not available, Camrelizumab concentrations may be determined as follows:
2. Calculate the mean OD of duplicate samples.
3. On graph paper plot the mean OD of the standards (y-axis) against the concentration (ng/ml) of Camrelizumab (x-axis). Draw the best fit curve through these points to construct the standard curve. A point-to-point construction is most common and reliable.
4. The Camrelizumab concentrations in unknown samples and controls can be determined by interpolation from the standard curve.
5. Multiply the values obtained for the samples by the dilution factor of each sample.
6. Samples producing signals higher than the 40 ng/ml standard should be further diluted and re-assayed.

Typical Results:

Wells	Calibrators		A450 nm
A1,2	**Negative Diluent Blank**		0.01
B1,2	2.5 ng/ml	**Standard**	0.25
C1,2	5 ng/ml	**Standard**	0.48
D1,2	10 ng/ml	**Standard**	0.95
E1,2	20 ng/ml	**Standard**	1.81
F1,2	40 ng/ml	**Standard**	3.03
G1,2	**Control** [11 – 21 ng/ml]		1.47

Positive Control Result = **15.8** ng/ml



PERFORMANCE CHARACTERISTICS

Specificity

The plate is coated with purified recombinant PD-1, expressed in CHO cells, to which Camrelizumab binds with high affinity. Other antibodies or binding proteins may also bind to the PD-1-antigen coated plate; however, the Anti-Human IgG4-HRP conjugate will not bind to non-human antibodies or non-antibody human serum proteins. Therefore, the assay is highly specific for measuring Camrelizumab activity.

Precision

Samples containing low, medium, and high concentrations of Camrelizumab were assayed multiple times in the same assay (n=10) to provide within-assay precision and as duplicates in multiple assays (n=5) to obtain between-assay reproducibility. Coefficient of variations were calculated for the concentrations using a 4PL curve-fitting program.

Results

Camrelizumab concentrations were measured with good within-assay and between-assay (**1.2** to **5.5** %CV) reproducibility.

Sample	Camrelizumab ng/ml	Intra-assay %CV	Inter-assay %CV
High Concn	30	**3.2**	**1.2**
Medium Concn	16	**2.8**	**2.0**
Low Concn	3.75	**1.4**	**5.5**

Recovery

Camrelizumab was spiked into human serum diluted 1:50 in Sample Diluent at 5 and 20 ng/ml, and assayed for anti-PD-1 activity. **Recovery** was calculated comparing the observed (O) values to the expected (E) values for each diluted sample. All serum and plasma samples contained 0 Camrelizumab (E).

Results

Camrelizumab was recovered from normal human serum diluted 1:50 with an average of **97%** (91 – 104%). See Limits of the Assay.

Sample	% Recovery (O/E x 100)					
	O	E	O/E	O	E	O/E
Human A	4.68	5	**94**	19.7	20	**98**
Human B	5.01	5	**100**	18.9	20	**94**
Human C	5.07	5	**101**	19.4	20	**97**
Human D	5.19	5	**104**	18.7	20	**93**
Human E	4.96	5	**99**	19.3	20	**97**
Human F	4.86	5	**97**	18.3	20	**91**
Human G	4.94	5	**99**	18.8	20	**94**

QUALITY CONTROL

Reagents: Accurate and reproducible assay results rely on proper storage, handling, and control of reagent and sample temperature. Store all reagents as indicated and warm to room temperature only those to be used in the assay. Shelf-life of the critical reagents and samples will diminish with extended exposure to non-refrigeration, resulting in inaccurate assay results. All solutions should be clear. Cloudiness or particulates are indications of reagent contamination or instability and may interfere with proper performance of the assay. Do not use.

Sample Controls: A Positive Control is provided with the kit, assigned with a Camrelizumab concentration value range. Recovery in this range is an indicator of proper assay performance. Each lab should also assay internal control samples, which represent the lab's expected sample population and that are maintained stabilized. A Sample Diluent blank should also be run; OD should be <0.3 and lower than 2.5 ng/ml Standard OD.

Standard Curve: The signal generated by the standards should be continuously increasing in OD from the lowest Standard to the highest Standard, with a difference greater than 1.2 OD. Non-uniform or low signals may indicate problems with technique, protocol directions, and/or reagent preparation, use, or stability. Do not rely on results generated from an assay with these issues.

Technique: Accurate and reproducible assay results rely on good lab technique regarding pipetting, plate washing, and handling of samples and reagents.

Equipment: Precision of results relies on uniform and effective washing techniques; an automatic washer may be used. ELISA reader and pipettes should be properly calibrated.

LIMITS OF THE ASSAY

1. The assay measures Camrelizumab activity, i.e., antibody that actually binds to the PD-1-antigen coated plate relative to Camrelizumab standards that are presumed to be 100% active IgG. Factors in the sample that diminish Camrelizumab plate-binding, e.g., PD-1 antigen or other Camrelizumab binding molecules, may reduce apparent Camrelizumab concentration in the assay (see **Recovery,** page 6).

2. Assays that measure Camrelizumab mass concentration may not have a tight correlation with the Camrelizumab activity assay, e.g., full Camrelizumab recovery may be determined by different assay factors.

3. **Recovery** in fresh, individual human or animal serum or plasma samples may differ from stored serum which has been used in the current validation studies.

4. As with all therapeutic antibodies, there is a potential for **immunogenicity with Camrelizumab**. Anti-drug (Camrelizumab) antibodies (ADAs) have been observed in treated patients. However, when large amounts of a monoclonal immunoglobulin are continually encountered in circulation, the host may mount significant 'anti-idiotypic' response = anti-Camrelizumab antibodies. Such antibodies might be expected to diminish the effectiveness of Camrelizumab as a drug and perhaps have other metabolic consequences. Such ADAs may lower Camrelizumab detection and quantification in the anti-PD-1 assay.

Instruction Manual No. M-200-585-CAM

Camrelizumab (anti-PD-1 humanized IgG) ELISA Kit

Cat. #200-585-CAM, 96 Tests

For Quantitation of Active Camrelizumab in Serum/Plasma and other Biological Solutions

For research use only (RUO), not for diagnosis, cure, or prevention of the disease.

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ELISA Kit Components	Amount	Part
PD-1 Antigen Coated Microwell Plate	8-well strips (12)	200-561
Camrelizumab Positive Control	0.65 ml	200-587PC
Camrelizumab Standard 2.5 ng/ml	0.65 ml	200-587B
Camrelizumab Standard 5 ng/ml	0.65 ml	200-587C
Camrelizumab Standard 10 ng/ml	0.65 ml	200-587D
Camrelizumab Standard 20 ng/ml	0.65 ml	200-587E
Camrelizumab Standard 40 ng/ml	0.65 ml	200-587F
Anti-Human IgG4-HRP Conjugate (100X)	0.15 ml	H-HuG4-612
Sample Diluent Concentrate (20x)	10 ml	SD20T
Wash Solution Concentrate (100X)	10 ml	WB-100
TMB Substrate	12 ml	80091
Stop Solution	12 ml	80101
Product Manual	1 ea	M-200-585-CAM