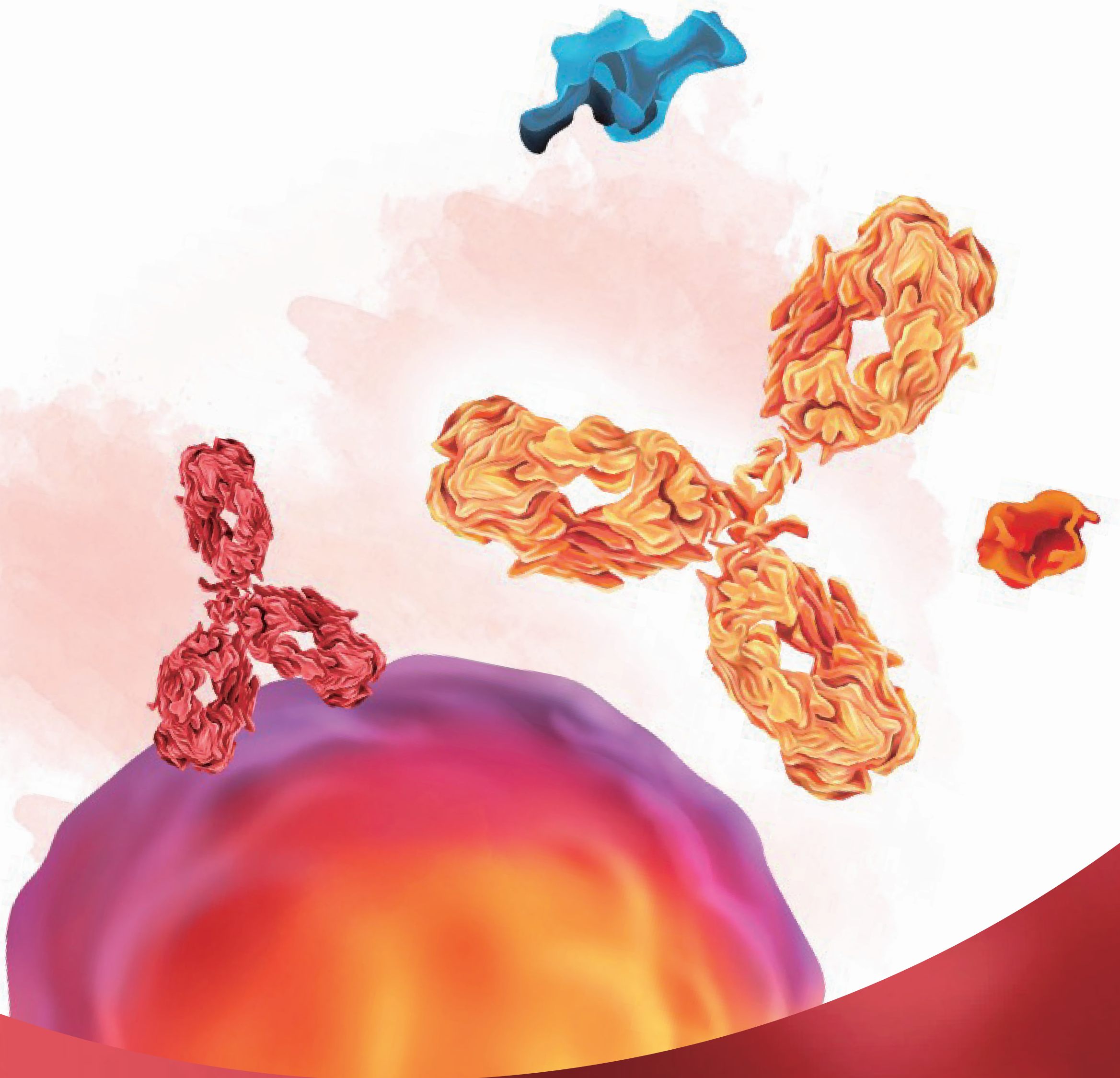
BIOSYSTEMS
Acro

ELISA Assay Kits for Quantitative Analysis of Therapeutic Antibodies in Serum Samples

Low Background | High Batch-to-batch Consistency | High Stability



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Introduction

Pharmacokinetics (PK) is a branch of science dedicated to the quantitative analysis of absorption, distribution, metabolism and excretion of drug molecules within the body of a living organism. All pre-clinical and clinical studies include the measurement of serum drug concentration, both in animals and in patients, at different points after drug administration. The result is an important indicator of the drug's pharmacokinetic properties and is pertinently relevant to dosing recommendations.

Case Study

The booming market of biologic drugs, driven by a flurry of success with monoclonal antibodies, brings the need for standard high throughput assays to evaluate the content of mAbs in serum samples. There are several types of assay designs based on different principles. The table below listed three most common ELISA setups for serum antibody detection, and their general advantages and disadvantages. To evaluate their performance in real applications, we tested them in the following case studies measuring the concentration of anti-PD1 mAb in serum samples.

Method	Coating	Sample	Secondary Antibody	Advantage	Disadvantage	Case Studies
indirect assay	antigen	serum	goat anti-human IgG	simple	high background	case I
antibody-directed competitive assay	antigen	serum with labeled competition antibody	SA-HRP	low background	additional reagent/labeling required	case II
ligand-directed competitive assay	natural ligand for the antigen	serum with labeled PD-1	SA-HRP	low background	narrow detection range;	case III

Case I: Indirect ELISA

Equipment：BMG CLARIOstar microplate reader
Sample：Serum samples containing a monoclonal PD-1 antibody
Main Reagents：
Recombinant Human PD-1 protein (Cat. No. PD1-H5221, ACROBiosystems, Newark, DE, USA)
Peroxidase AffiniPure Goat Anti-Human IgG, Fcγ Fragment Specific (Cat. No. 109-035-098, Jackson Lab, Bar Harbor, ME, USA)
Protocol:
1. Coat the microplate with 0.1μg/well rhPD-1 for 16hr;
2. Prepare serial sample dilutions (1:2);
3. Wash the plate;
4. Add samples 100μl onto the plate;
5. Wash the plate;
6. Add HRP-conjugated Goat Anti-Human IgG 0.05μg/ml;
7. Add TMB for colorimetric detection.

Result：
To avoid unspecific binding, all serum samples was diluted by a factor of 1000 before assays. The detection range is 0.19-6.25μg/mL, and the sensitivity is 0.19μg/mL (Figure 1).

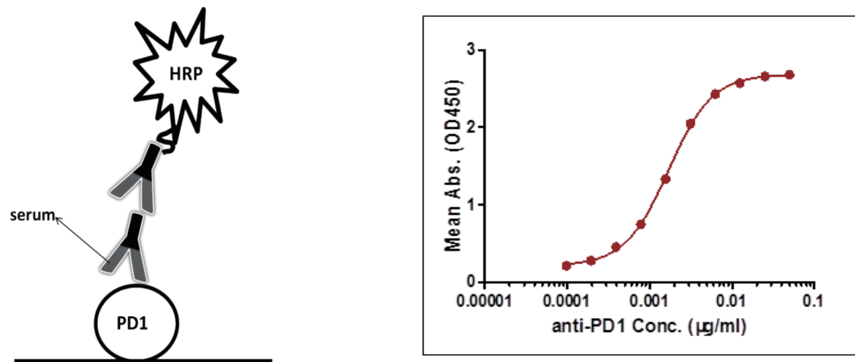


Figure 1. Detection of PD-1 antibody by indirect ELISA.

Case II: Antibody-directed competitive ELISA

Equipment: BMG CLARIOstar microplate reader
Sample: Serum samples containing a monoclonal PD-1 antibody
Main Reagents:
ELISA Assay Kit for Anti-PD-1 h-mAb in Human Serum (Cat. No. EPH-V1, ACROBiosystems, Newark, DE, USA)
Simplified Protocol:
1. Coat the microplate with 0.1µg/well rhPD-1 for 16hr;
2. Prepare serial sample dilutions (1:2);
3. Mix samples with the biotinylated PD-1 antibody provided in the kit to a final concentration of 10%;
4. After washing, add the mixed samples 100µl from step 2 to the wells;
5. After washing, add HRP conjugated Streptavidin 0.1µg/ml;
6. Add TMB for colorimetric detection.

Result:
The detection range is 0.78-25µg/mL and the sensitivity are 0.78µg/mL (Figure 2).

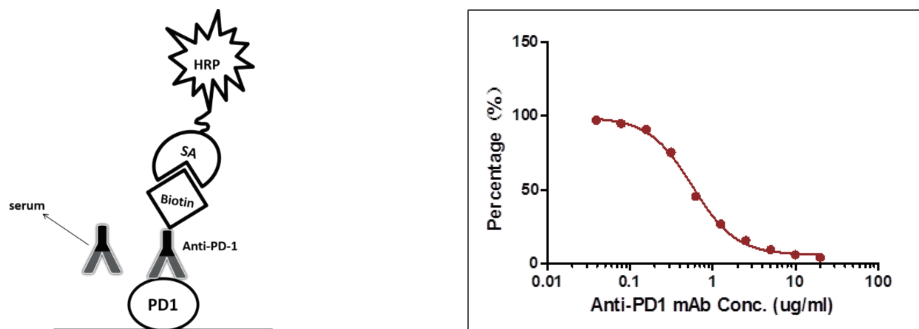


Figure 2. Detection of PD-1 antibody by antibody-directed competitive ELISA.

Case III: Ligand-directed competitive ELISA

Equipment: BMG CLARIOstar microplate reader
Sample: Serum samples containing a monoclonal PD-1 antibody
Main Reagents:
Recombinant human PD-L1-Fc protein (Cat. No. PD1-H5258, ACROBiosystems, Newark, DE, USA)
Biotinylated human PD-1 (Cat. No. PD1-H82F2, ACROBiosystems, Newark, DE, USA)
HRP-conjugated Streptavidin Protein (Cat. No. 21126, Thermo Fisher Scientific)
Simplified Protocol:
1.Coat the microplate with 0.2µg/well rhPD-L1 for 16hr;
2.Prepare serial sample dilutions (1:2), and mix with biotinylated PD-1 to a final concentration of 10%;
3.After washing, add the samples 100µl from step 2 to the wells;
4.After washing, add HRP conjugated Streptavidin 0.1µg/ml;
5.Add TMB for colorimetric detection.

Result:
The detection range is 1.565-6.25µg/mL and the sensitivity are 1.565µg/mL (Figure 3).

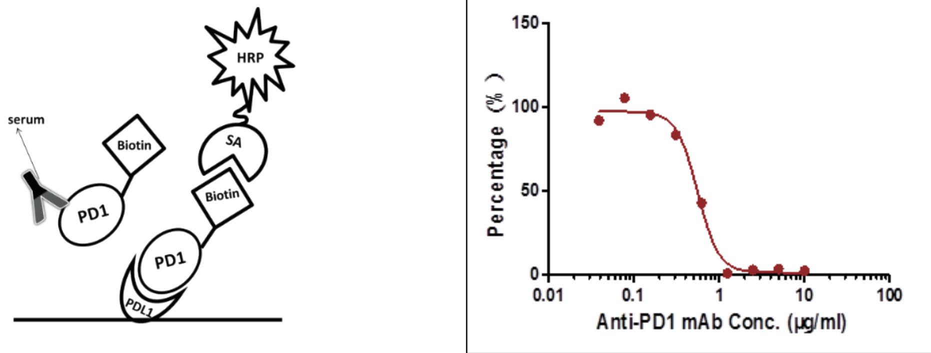


Figure 3. Detection of PD-1 antibody by ligand-directed competitive ELISA.

Methods Comparison

Case	Detection Range (μg/mL)	Sensitivity (μg/mL)	Specificity
Case I: Indirect ELISA	0.19-6.25	0.19	High background
Case II: Antibody-directed competitive ELISA	0.78-25	0.78	Very low background
Case III: Ligand-directed competitive ELISA	1.565-6.25	1.565	Very low background

Among the aforementioned three assay designs, the ligand-directed competitive ELISA (Case III) has the narrowest detection range and lowest sensitivity, indicating that it's not the best solution for the application. The sensitivity for the indirect ELISA is the best. However, the use of goat anti-human IgG as secondary antibody results in high background due to unspecific binding, and therefore require pre-dilution before analyses (Fig. 4A). On the other hand, the antibody-directed competitive ELISA uses HRP-conjugated Streptavidin for secondary detection, which minimize the background interference (Fig. 4B). In addition, it also has the widest detection range among the group, although the sensitivity is lower than the indirect method.

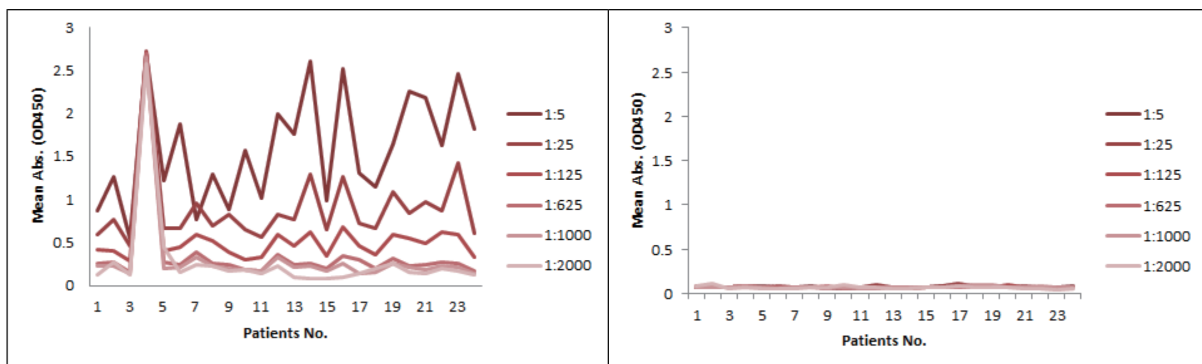


Figure 4. Comparison between Indirect ELISA and antibody-directed competitive ELISA for PD1 mAb detection in patient samples. A: Indirect ELISA; B: antibody-directed competitive ELISA.

In most applications, the background issue is a bigger concern than detection sensitivity, as a detection sensitivity of less than 1μg/ml is already good enough. Therefore, we opt to use the antibody-directed competitive ELISA for the PK kits.
We have developed kits for the studies of mAbs against CTLA-4, PD-1, and HER-2, respectively, in both human serum samples, and mouse and monkey serum samples.

Product Features

Low Background

The serum samples contain many factors that may potentially interfere with the indirect ELISA result. This is the major reason for the background issue (Fig. 4A). Therefore, a series of testing need to be performed to determine the minimum required dilution (MRD) before an experiment can be conducted. This can be time consuming and the results can vary. On the other hand, the competitive ELISA method we employed for our kit does not have a background issue. As shown in Fig 4B, dilutions up to 1:5 does not produce any background at all.

High Batch-to-Batch Consistency

We install rigorous quality control program to ensure the lot-to-lot consistency of our products. Every batch of products are analyzed against our internal standards using various analytical methods. The product will be released only if all standards are met.

High Stability

All kits components are analyzed for their stability using accelerated testing method. Based on the results shown in Fig. 5, the products can be stored at -80 for 4-6 months.
The assay components are also tested after one or two freeze-thaw cycles. No significant activity loss was observed under either condition.

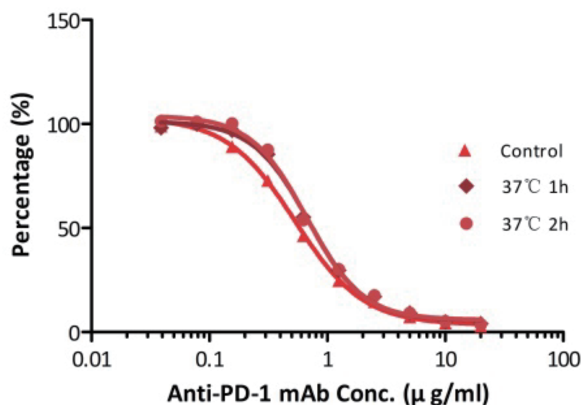


Figure 5. ELISA using Anti-PD-1 h-mAb kit Human Serum (Cat. EPH-V1). The samples were incubated at 37°C for 2 hours after reconstitution. No significant loss of activity was observed.

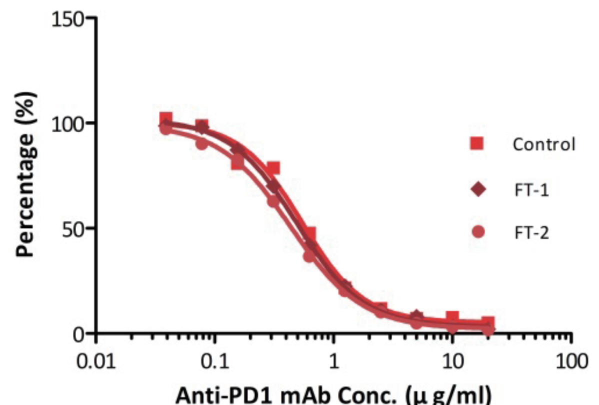
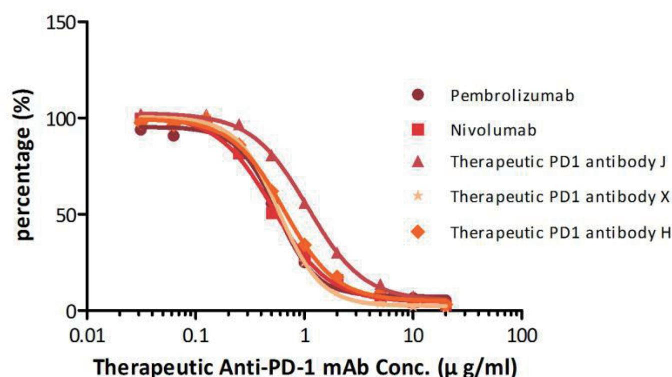


Figure 6. ELISA using Anti-PD-1 h-mAb kit Human Serum (Cat. EPH-V1). The samples were subjected to zero, one, and two rounds of freeze-thawing cycles,respectively. No significant loss of activity was observed.

Application

The assay kits can be used for studies of any mAbs share similar binding domain as our tracer biotinylated antibody. For example, with our Anti-PD-1 mAb kit for human serum samples (Cat. EPH-V1), we have successfully measured five different anti-PD1 mAbs that are either on the market already or being tested in clinical trials.



Name	Pembrolizumab	Nivolumab	Therapeutic PD-1 Antibody J, Human IgG4	Therapeutic PD-1 Antibody X, Human IgG4	Therapeutic PD-1 Antibody H, Human IgG4
Detection Range (μg/ml)	0.03125-20	0.03125-20	0.03125-20	0.03125-20	0.03125-20
Sensitivity (μg/ml)	0.15625	0.15625	0.15625	0.15625	0.15625
%Recovery	88-113	92-114	86-114	96-112	87-106

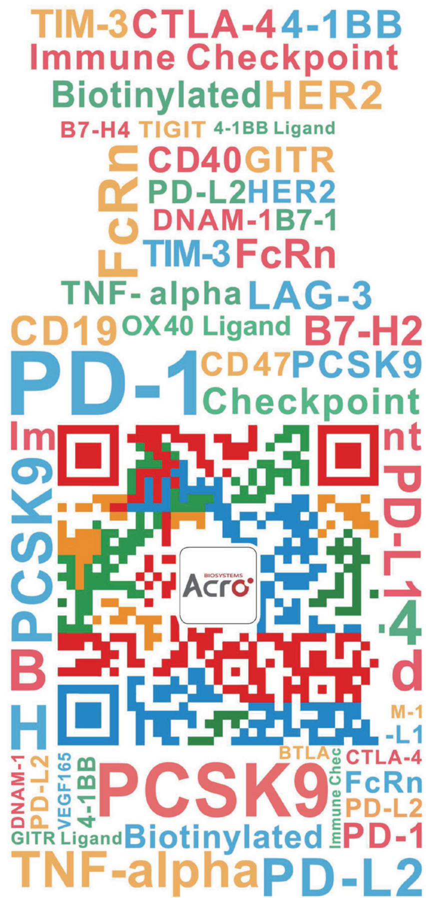
Figure 7. Determination of serum drug concentration for five PD-1 therapeutic antibodies using PD-1 ELISA Kit

Product List

Cat.	Product Description	Size
EPH-V1	ELISA Assay Kit for Anti-PD-1 h-mAb in Human Serum	96/480 tests
EPM-V1	ELISA Assay Kit for Anti-PD-1 h-mAb in Mouse Serum	96/480 tests
EPC-V1	ELISA Assay Kit for Anti-PD-1 h-mAb in Monkey Serum	96/480 tests
EHH-V1	ELISA Assay Kit for Anti-HER-2 h-mAb in Human Serum	480 tests
EHM-V1	ELISA Assay Kit for Anti-HER-2 h-mAb in Mouse Serum	480 tests
EHC-V1	ELISA Assay Kit for Anti-HER-2 h-mAb in Monkey Serum	480 tests
ECH-V1	ELISA Assay Kit for Anti-CTLA-4 h-mAb in Human Serum	96/480 tests
ECM-V1	ELISA Assay Kit for Anti-CTLA-4 h-mAb in Mouse Serum	96/480 tests
ECC-V1	ELISA Assay Kit for Anti-CTLA-4 h-mAb in Monkey Serum	96/480 tests

Coming soon

- ELISA Assay Kit for Adalimumab in Serum
- ELISA Assay Kit for Bevacizumab in Serum
- ELISA Assay Kit for Rituximab in Serum
- ELISA Assay Kit for Cetuximab in Serum



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