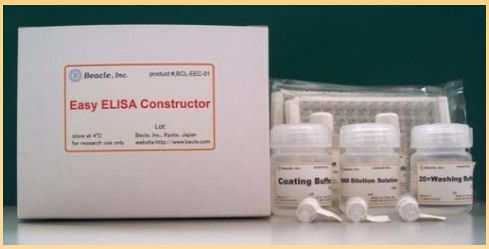
Beacle, Inc.

For Research Use only

ELISA construction and evaluation for antibodies

Easy ELISA Constructor (ab)

For quick construction of antibody-detecting ELISA and quick and highly sensitive evaluation of antibodies



It is often necessary to examine the antigen-binding activity of antibodies. Easy ELISA constructor (ab) is designed to quickly and easily construct antibody-detecting ELISA and quick and sensitive evaluation using the constructed ELISA by using our world-only one MAD (Multi-Antibody Detection) technology. All you need is antigens and standard apparatus. Using the detailed Manual, beginners can easily build ELISA and estimation of antibody's dilution factor.

The kit is also used for highly increasing sensitivity of antibody-detecting ELISA using HRP-labeled secondary antibody

Features

1. Easy to construct ELISA to evaluate antigen-binding activity of IgGs.
2. Can be used for IgG from various kind of animal species. *
3. The constructed ELISA is highly sensitive and requires only 90 min for a run.
4. The combination use with HRP-labeled antibody increases sensitivity more.
5. All needed is antigens to construct ELISA.
6. Estimation of dilution factor possible from the result of attached positive control,
7. Possible to highly increase the sensitivity of antibody-detecting ELISA using 2nd antibody.

*: Basic type (BCL-EEC-01) can detect IgG of rabbit, guinea pig, pig, dog, cat, monkey, human (However, some IgG subclasses have weak detection). There are other things for mouse and rat.

【Kit Composition】 Essentially all reagents except for antigen are provided (the detail in back page)

【Price】

Product #	Name	Content
BCL-EEC-01	Easy ELISA constructor (ab)	1 kit (192 tests)
BCL-EEC-M1	Easy ELISA constructor for Mouse (ab)	1 kit (192 tests)
BCL-EEC-R1	Easy ELISA constructor for Rat (ab)	1 kit (192 tests)

A kit contains two 96 well plates. Kit supply with less expensive and limited functions possible for business use,

【Related products】

Product #	Name	Content
BCL-TMB-01	TMB solution (1 reagent type)	250mL
BCL-TMB-21	TMB solution (2 reagent type)	250mL each
BCL-PIK-01	Peptides immobilizing kit	3 trials

【Contact to】

Beacle, Inc. (manufacturer and distributor)
14-1 Yoshida-Kawaracho,Sakyo-ku Kyoto,
606-8305 Japan
website: www.beacle.com
E-mail : information@beacle.com

【Principle and the ways to use the kits 】

The kit determines the antibodies bind to coated antigens. Antibodies (IgGs) are detected by our original antibody-detecting probe, MAD reagent . The MAD reagents enhances the sensitivity and minimize the time required for an assay.

For the use to evaluate the anitgen-binding activity of antibodies

Step 1:establishment of assay conditions
Step 2:Sample assay and evaluation
If the data exist concerning to the assay condition of the sample IgG, Step 1 may be omitted.
Each step requires 90 min at the minimum, So that all the procedure can be finished within half of a day.
An assay process with the least time schedule is shown below.
①Antigen coating:20 min ⇒ ②Blocking:15 min ⇒ ③Reaction of antibody with antigen: 20 min ⇒ ④MAD reaction with antibody: 20 min ⇒ ⑤Chromogenic reaction: 15min ⇒ ⑥Reading total: 90 min *
(if you need much higher response, use MAD reagents with HRP-labeled 2nd antibody.)
*: Time do not includes washing and sample preparation etc. Sensitivity may be higher with longer incubation time

For the use to elavate sensitivity of antigen-detecting ELISA

In the assay system where sample antibody bound to coated antigen is detected by HRP-labeled IgG (2nd antibody), the kit can used to elevate sensitivity of the antigen detection. With the combination use of MAD reagent with the 2nd antibody, the sensitivity may be elevated at maximum 100-fold. For more information see the manual or visit our website.

【Kit composition】

MAD Reagent (HRP labeled)	1	tube	Chromogenic reagent (2 solution type)	2	bottle
Antigen coating buffer	1	bottle	3X conc. blocking buffer	1	bottle
20X conc. washing buffer	1	bottle	Stop solution	1	bottle
MAD reaction buffer	1	bottle	96well microplate (split type)	2	plates
Control antigen	1	tube	Control antibody (rabbit)	1	tube

The composition may be changed without notification.
* In place of positive antibody (for rabbit), positive antibody (for mouse) and mouse sensitizer for mouse are included, for rats positive antibody (for rat) and rat sensitization reagent are included as constituents .

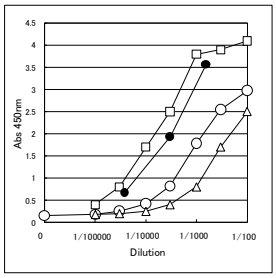
【Experimental Example】

● Evaluation of IgGs (sample: three mouse sera raised against GFP)

Pos. Control		Antiserum				
antibody		antibody	Coating antigen(μ g/mL)			
Dilution		Dilution	0	1	0.1	0.01
1/100	2.600	1/100	0.113	4.457	4.500	3.320
1/1000	1.544	1/1000	0.116	4.457	1.857	1.680
1/10000	0.501	1/10000	0.131	1.264	0.402	0.302
BL	0.151	BL	0.139	0.141	0.144	0.148

Step 1 Establishment of assay condition

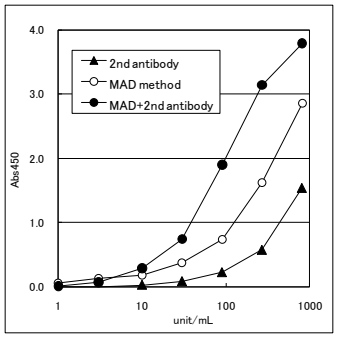
One sample was used for examining assay conditions at indicated coating antigen conc. and diiution shown in the table.
Interpretation
1. Positive control OK ⇒ ELISA system works well.
2. The best antigen dilution is to be 0.01 μg/mL (minimal conc. with enough response)
3. The dilution range to be 100 to below 1/10000 → go to next step



Step 2 Sample evaluation

Evaluated three anti-sera based on the condition determined in STEP 1, and obtained the result.
1. All three sample + control showed good dose-response relationship.
2. One serum (□) is nearly equal to control (●). The one (○) used for Step 1 was about 1/3 of control. The rest was below 1/10 of control.
3. (estimation) According to relative response to control, the highest serum may be used at 1/1000 to 1/10000, and the medium 1/100 to 1/1000 dilution for immunoassays.

● Sensitivity enhancement of antibody-detecting ELISA (sample: urinary anti-leishmania antibody)



Urinary antibody against Leishmania
Comparing to the 2nd antibody method, replace of 2nd antibody to MAD reagents increase about 2 to 3-fold increase of sensitivity. The comobination use 2nd antibody and MAD reagent increased the sensitivity about 50-fold.

(The study was conducted with Prof. Itoh of Aichi Medical Univ.)