ONEPot Immunoassay Kit <OpenGUS Method>

Cat. # DS850	ONEPot Immunoassay Kit, Fluorescent <OpenGUS Method>	
	Recombinant GUS mixture	100 U
	Reaction Buffer	50 ml
	Fluorescent Substrate	5 ml

Cat. # DS860	ONEPot Immunoassay Kit, Colorimetric <OpenGUS Method>	
	Recombinant GUS mixture	100U
	Reaction Buffer	50ml
	Colorimetric Substrate	5 ml

This is a kit for constructing an homogenous immunoassay using your antibodies, based on the OpenGUS Immunoassay by mutant β-glucuronidase (GUS).

This product is commercialized by BDL based on the research results of Dr. Hiroshi Ueda, Dr. Tetsuya Kitaguchi, Dr. Bo Zhu and co-workers of Tokyo Institute of Technology (at the time of research)

Storage conditions

−80 ℃

Expiration date

6 months after receiving the item

Overview

- Antigens are sandwiched and detected using two antibodies of your choice.
- Unlike ELISA, there is no need to immobilize antibodies on plates or wash wells.
- Just by mixing, the antibodies and recombinant GUS will form an affinity bond.
- Proteins that stably form multimers may be possible to be detected with only one type of antibody.
- Fluorescence measurement kit (Cat. #DS850) and absorption measurement kit (Cat. #DS860) are avilable.

Notes

- We recommend samples containing 3 μg/ml or more of the target protein.
  (Assuming that the sample is diluted 100 times and target protein is measured as 30 ng/ml)
- The pair of mouse IgG1 that can sandwich antigens are required.
  (When the antigen forms a multimer, there are cases where the antigen could be detected using only one type of antibody. (Lactoferrin, CIAP, CRP, AAV capsid shown in the Application below))
- Signal intensity can be significantly affected by the composition of the sample solution.
- Detection sensitivity varies depending on antigen and antibody.
- Samples containing a large amount of immunoglobulin such as human serum/plasma/blood cannot be used because the background will be high.

Reagent and equipment to be prepared by the user

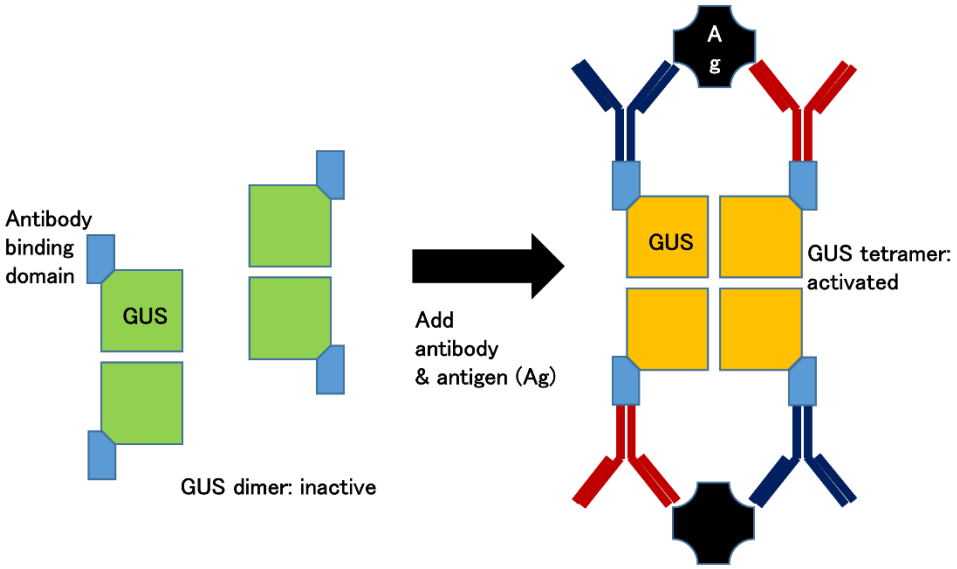
- Mouse IgG1 antibody pair that can sandwiching antigens
- Purified antigen for standard curve
- 96 well plate
  Fluorescence (Cat. #DS850): black plate
  Absorbance (Cat. #DS860): clear plate: Nunc™ Edge™ 96-Well, Non-Treated, Flat-Bottom Microplate (Thermo Scientific #267427) is recommended * This plate Allows the outside of the wells to be filled with water, reducing temperature differences between wells.
- Microplate reader
  Fluorescence (Cat. #DS850): Excitation 340 nm / Emission 450 nm
  Absorbance (Cat. #DS860): Main wavelength 405nm / Reference 660nm
- Multichannel pipettor

Kit composition

Recombinant GUS mixture

β -glucuronidase (GUS) is an enzyme that hydrolyzes β-glucuronide to β-glucuronic acid. E. coli GUS exhibits activity by forming a tetramer. The mutant GUS used in this product has reduced affinity between monomers, and although it forms dimers, it rarely forms tetramers.

Recombinant GUS mixture, a component of this product, is a mutant GUS fused with antibody binding domains. Dimers of mutant GUS link together through antigen-antibody reactions and forcefully form tetramers. By measuring the activity of GUS, antigen concentration is estimated.



Reaction Buffer

A phosphate base buffer optimized for the ONEPot Immunoassay Kit. Sample dilution is also done in Reaction Buffer.

Substrate

Fluorescent substrate (Cat. #DS850) or colorimetric substrate (Cat. #DS860). Add substrate to 1/5 of the total amount of the reaction system.

Procedure

Reaction Buffer is frozen at −80℃. Thaw at room temperature or in water (avoid getting water to the lid), mix well, make sure there is no solid material, and leave on ice until use.
Substrate is frozen at −80℃. Thaw at room temperature in a light-blocking bag. Dispense the amount to be used 30 minutes before addition and keep it at room temperature protected from light.

1. Dispense the required amount of Reaction Buffer and leave it on ice.
2. Melt the Recombinant GUS mixture on ice and vortex gently *1
3. Prepare premix as below

Premix (per 1 well)

Recombinant GUS mixture	1U *2
Mouse IgG1 (No. 1)	X µl (75 ng) *3
Mouse IgG1 (No. 2)	Y µl (75 ng)
Reaction Buffer	Z µl
	140 µl / well

4. Plate the 96 well plate on ice *4
5. Dispense 140 µl of premix into each well *5
6. Dilute the sample with Reaction Vuffer (recommended: 10 times or more) *6 *7 *8 *9
7. Add 20 µl of diluted samples to each well
8. 4℃ for 60 minutes *10
9. Incubate at room temperature (approximately 25℃) for 10 minutes *11
10. Add 40 µl of substrate to each well *12 *13
11. Fluorescence (Cat. #DS850) : Incubate at 25℃ for 60 minutes, protected from light.
    Colorimetric (Cat. #DS860 : Incubate at 37℃ for 60 minutes, protected from light. (The reaction solution turns yellow)
12. Measure the Fluorescence intensity or absorbance.
    Fluorescence (Cat. #DS850): Excitation 340 nm / Emission 450 nm
    Colorimetric (Cat. #DS860) : Main wavelength 405nm / Reference 660nm

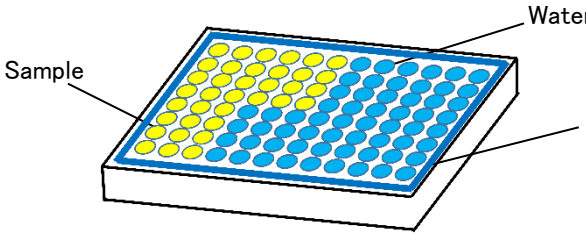
＊1 Return the Recombinant GUS mixture to −80℃ immediately after use.

＊2 The amount of Recombinant GUS mixture per 1U varies depending on the lot. Please check the tube label.

＊3 When the antigen forms a multimer, there are cases where the antigen could be detected using only one type of antibody (Lactoferrin, CIAP, CRP, AAV capsid shown in the Application below). In that case, please use 150 ng/well of antibody.

＊4 When placing the plate on ice, place aluminum foil or plastic wrap between the plate and ice, to prevent the bottom of the plate from getting wet.

＊5 Reactions are affected by temperature. Especially at 37℃, it is important that the temperature of all wells rises simultaneously. Nunc™ Edge™ 96-Well, Non-Treated, Flat-Bottom Microplate (Thermo Scientific #267427) recommended for absorbance measurements is designed so that the outside of the wells can be filled with water, allowing the temperature difference between each well is smaller. Even when using this plate, the measurement results will be more accurate if 200 µl of water added to the unused wells.



Nunc™ Edge™ 96-Well, Non-Treated, Flat-Bottom Microplate (Thermo Scientific #267427) recommended for absorbance measurements is designed so that the outside of the wells can be filled with water.

＊6 At an appropriate antigen concentration, the signal will increase in an antigen concentration-dependent manner. But if the antigen is at a higher concentration than the appropriate concentration, the signal may decrease. If the sample is measured for the first time, please serially dilute it. And use the point where the signal increases in a concentration-dependent manner to determine the concentration.

＊7 If the sample is not diluted sufficiently, it may affect the activity of GUS. Serially dilute the sample and use each result to calculate the antigen concentration using a standard curve. If the antigen concentrations calculated by multiplying by the dilution factor are about the same, the dilution is considered to be sufficient (see “Application 1” below). If you can prepare a sample that does not contain the target antigen, performing a Spike & Recovery test is also effective in determining the appropriate sample dilution concentration (see “Spike & Recovery test” below).

＊8 Biological samples may have GUS activity. Mix only the sample, Substrate, and Reaction Buffer to ensure that the sample itself does not have GUS activity. If the sample itself has GUS activity, signal obtained by the sample, Substrate, and Reaction can be subtracted as background.

＊9 By making the buffer composition in the dilution series used for the calibration curve equal to the buffer composition of the sample, measurements can be performed with fewer errors. e.g., if the sample is a column-purified protein, add elution buffer to the standard dilution series for the calibration curve.

＊10 If the Signal/Background is sufficiently high, the step of 60 minutes at 4℃ before adding Substrate can be omitted. However, in this case as well, work should be done on ice.

＊11 Bring the temperature close to the substrate reaction temperature. This is to avoid differences in temperature rise between wells when adding substrate.

＊12 We recommend using a multichannel pipettor to avoid misalignment of the timing of adding the Substrate. After adding Substrate, mix the solution by pipetting about 4 times.

＊13 If bubbles remain in the well, blow air to remove them.

FAQ

Question	Answer
We have a plate reader that can measure both fluorescence and absorbance. Which kit should I choose, Fluorescence (Cat. #DS850) or Colorimetric (Cat. #DS860)?	We recommend the Fluorescent kit (Cat. #DS850) as it tends to have slightly higher sensitivity.
There is no 25℃ incubator for fluorescent substrate reactions.	Cover the plate with a lid and aluminum foil to protect it from light, and reaction at room temperature.
The antibody contains sodium azide, is it okay?	We have confirmed that sodium azide at a final concentration of 0.1% has no major effect.
Is it possible to measure fluorescence using something other than Excitation 340 nm / Emission 450 nm?	We have confirmed that measurements can be performed with Excitation 360 nm and Emission 480 nm. However, the sensitivity will be slightly reduced.
Does absorbance measurement have to be at 405 nm?	It is possible around 405 nm. However, the absorbance will decrease slightly. Reference can also be measured around 660nm.
Can I use antibodies other than Mouse IgG1?	This product is optimized for mouse IgG1. We have confirmed that there are antibody clones with high background for Mouse IgG2a, IgG2b, IgG2c, and IgG3. Also confirmed that the background is high with Rabbit polyclonal antibodies.
Can anti-tag antibodies be used?	Anti-6xHis antibody cannot be used. We have not confirmed any other anti-tag antibodies.
What concentration of protein can be measured?	The measurable range varies depending on the antigen and antibody. Please refer to the table below regarding "Antigen concentration range for which the calibration curve was created".

Antigen concentration range for which the calibration curve was created (Antigen concentration during substrate reaction)

antigen	concentration
Lactoferrin	3 – 824 ng/ml (0.04 – 10 nM)
CRP	0.06 – 57 ng/ml (0.0025 – 2.5 nM)
Cryj1	3.4 – 117 ng/ml (0.08 – 3 nM)
C.I.A.P.	66 – 4200 ng/ml (0.47 – 30 nM)
AAV2 (empty capsids)	$9.4x10^{8}$ – $6.0x10^{10}$ copies / ml

Troubleshooting

Problem	Possible cause	Correspondence
low signal	−Low antigen concentration −Low antigen−antibody affinity	Increasing the substrate reaction time, and the 4℃ incubation time before substrate reaction, may increase the signal.
	Antigen concentration is too high	At an appropriate antigen concentration, the signal will increase in an antigen concentration−dependent manner. But if the antigen is at a higher concentration than the appropriate concentration, the signal may decrease. If the sample is measured for the first time, please serially dilute it. And use the point where the signal increases in a concentration−dependent manner to determine the concentration.
	Sample solution affects GUS activity	I If the sample is not diluted sufficiently, it may affect the activity of GUS. Serially dilute the sample and use each result to calculate the antigen concentration using a standard curve. If the antigen concentrations calculated by multiplying by the dilution factor are about the same, the dilution is considered to be sufficient (see "Application 1" below). If possible, add the same amount of sample preparation solution to each point of the standard used in the calibration curve as the sample contains. e.g., If the sample is a column−purified protein, add elution buffer to the standard dilution series for the calibration curve.
Signal /Background is low	−Antigen concentration −Low antigen−antibody affinity	Signal/Background may increase by extending the incubation at 4℃ to 2 hours before substrate addition .
Duplicated or triplicated data is inconsistent	Each well has different temperature	Adding 200 µl of water to unused wells will reduce the difference in temperature rise between each well. It may be useful to perform the reaction with the substrate at 25℃ instead of 37℃ in absorbance measurement. In this case, extend the reaction time.

Spike & Recovery test

The Spike & Recovery test can be performed when a "sample that does not contain the antigen of interest (blank sample)" can be prepared. This is a method to evaluate the antigen measurement error (% Recovery) (influence of sample solution on the system) by comparing [diluted blank sample with antigen of known concentration added (Spike)] and [Reaction Buffer with antigen of same concentration].

1. Prepare [diluted blank sample spiked with antigen] and [Reaction Buffer spiked with the same concentration of antigen]. The amount to be spiked is the concentration within the range of the calibration curve (usually around the middle point of the calibration curve).
2. Perform measurements using this product for [spiked diluted blank sample], [diluted blank sample only], [spiked Reaction Buffer], and [Reaction Buffer only].
3. Calculate % Recovery:
   % Recovery
   = ([spiked diluted blank sample] − [diluted blank sample only]) / ([spiked Reaction Buffe] − [Reaction Buffer only])
   × 100

   % Recovery is preferably in the range of 80−120%.

Applications

*The vertical axis of the calibration curve is the [Signal – Background] value

Application 1　Measurement of Lactoferrin concentration in saliva and tears

Lactoferrin in saliva and tears was measured using this product. The results were compared with those obtained using the ELISA kit.

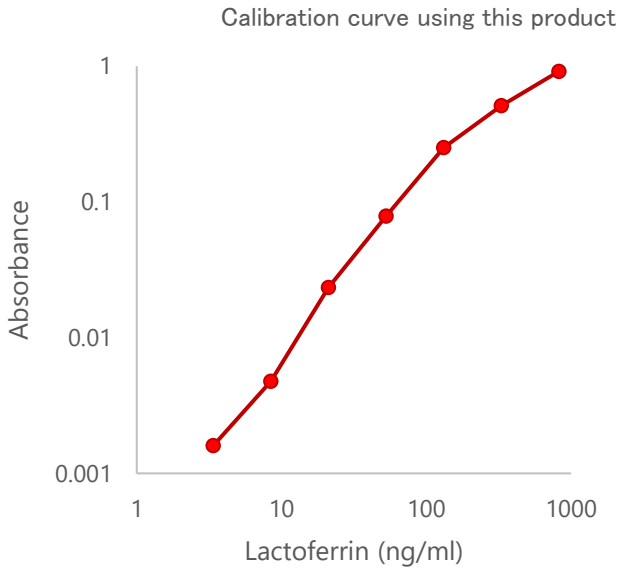
ELISA kit

	sample dilution	Lactoferrin conc. calculated from the calibration curve
saliva	1000 times	6.2 μg/ml
	2000 times	5.7 μg/ml
tears	200000 times	2158 μg/ml
	400000 times	2175 μg/ml

ONEPot Immunoassay kit

	sample dilution	Lactoferrin conc. calculated from the calibration curve
saliva	300 times	4.2 μg/ml
	600 times	4.4 μg/ml
tears	40000 times	1831 μg/ml
	80000 times	1734 μg/ml

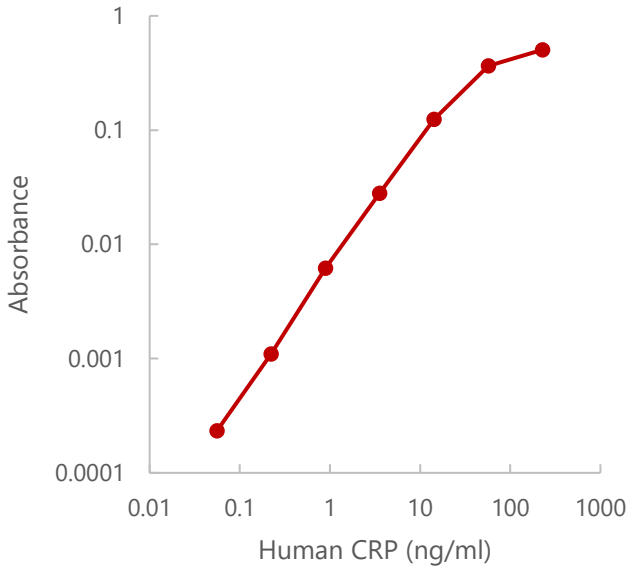
Similar result was obtained.



Antibody: Anti-Lactoferrin, Human, Mouse-Mono(1A1) (HyTest, #4L2-1A1)

* Detection was performed using only one type of antibody. Since Lactoferrin forms a multimer, we believe that only one type of antibody was required to detect Lactoferrin.

Application 2　Calibration curve obtained using purified human C－Reactive Protein

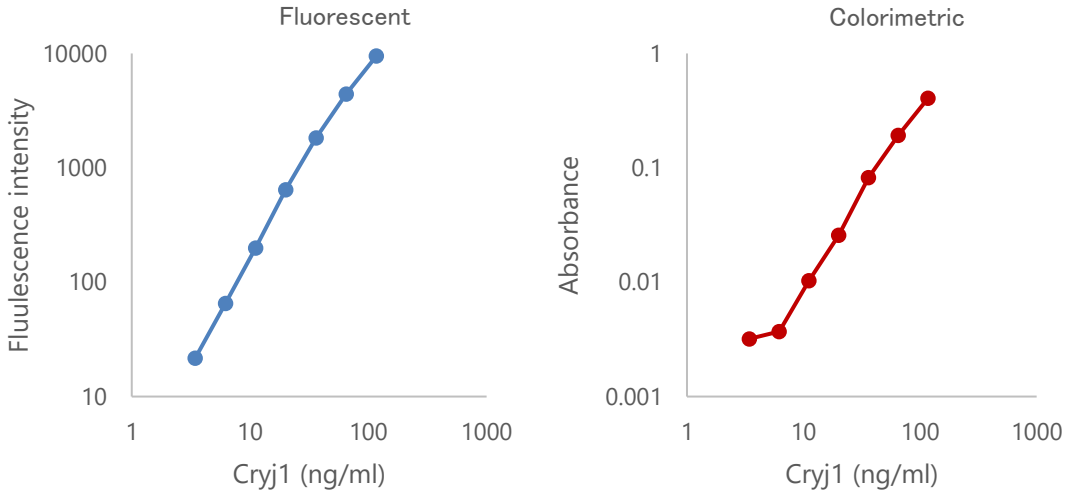


Antibody: Anti-CRP, Human, Mouse-Mono(C5) (HyTest #4C28-C5)

*Detection was performed using only one type of antibody. Since CRP forms a pentamer, we believe that only one type of antibody required to detect CRP.

*This product has a high background value in human serum, so it was not possible to measure CRP in human serum.

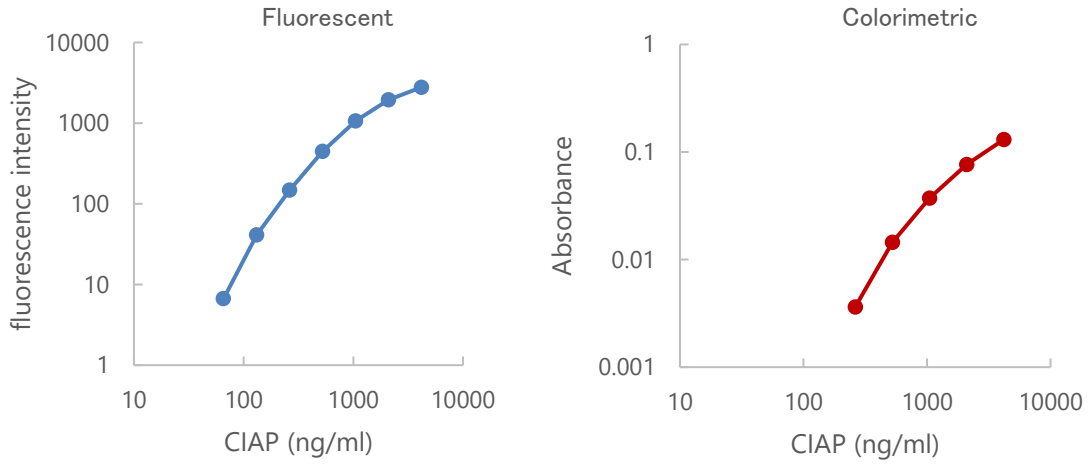
Application 3　Calibration curve obtained using purified Cry j 1 (cedar pollen antigen)



Antibodies: Anti-Cry j1 Mouse-Mono(013) (BDL #HBL-Ab-1-013) and Anti-Cry j1 Mouse-Mono(053) (BDL #HBL-Ab-1-053)

* Cry j 1 could be measured from Cry j 1 enriched samples, but the sensitivity was low to detect Cry j 1 from environmental samples.

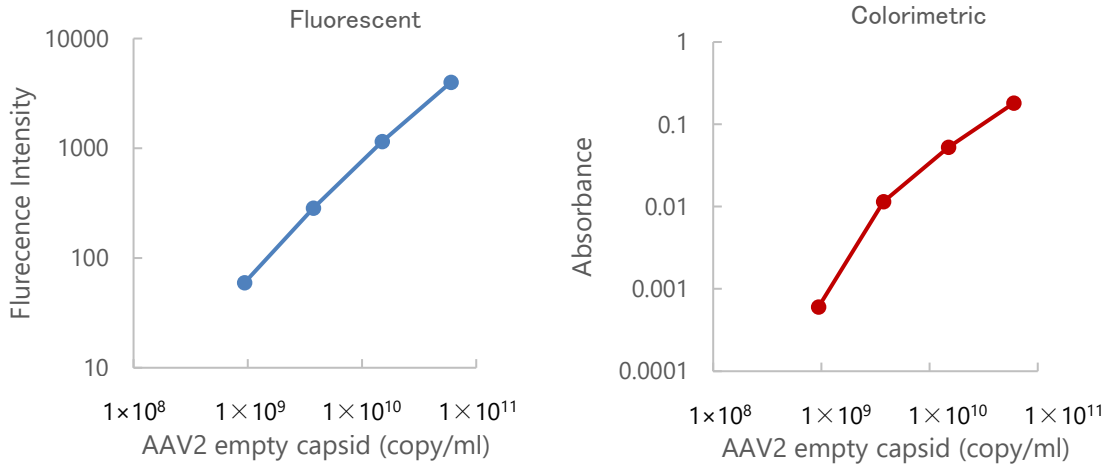
Application 4　Calibration curve obtained using purified Calf-Intestinal Alkaline Phosphatase



Antibody: Anti-Alkaline Phosphatase, Intestinal, Mouse-Mono(SPM372) (Novus Biologicals #NBP2-44961)

* Detection was performed using only one type of antibody. Because CIAP forms a dimer , we believe that only one type of antibody was required to detect CIAP.

Application 5　Calibration curve obtained using AAV2 empty capsids



Antigen: AAV2 empty capsids (PROGEN #66V020)

Antibody: Anti-AAV2 (intact particle) mouse recombinant, (A20R) (PROGEN #610298)

* Detection was performed using only one type of antibody. Since antigens aligned on the Capsid surface, we believe that only one type of antibody was required to detect AAV empty capsids.

References

1. J. Su, D. Jinhua, T. Kitaguchi, Y. Ohmuro-Matsuyama and H. Ueda. Noncompetitive homogeneous immunodetection of small molecules based on beta-glucuronidase complementation. Analyst 143, 2096-2101 (2018)
2. J. Su, C. Beh, Y. Ohmuro-Matsuyama, T. Kitaguchi, S. Hoon and H. Ueda. Creation of stable and strictly regulated enzyme switch for signal-on immunodetection of various small antigens. J. Biosci. Bioeng. 128, 677-682 (2019)
3. J. Su, T. Kitaguchi, Y. Ohmuro-Matsuyama, T. Seah, F.J. Ghadessy, S. Hoon and H. Ueda. Transmembrane signaling on a protocell: Creation of receptor-enzyme chimeras for immunodetection of specific antibodies and antigens. Sci. Rep. 9, 18189 (2019)
4. B. Zhu, C. Qian, H. Tang, T. Kitaguchi and H. Ueda. Creating a thermostable beta-glucuronidase switch for homogeneous immunoassay by disruption of conserved salt bridges at diagonal interfaces. Biochemistry 62, 309-317 (2023)
5. B. Zhu, Y. Yamasaki, T. Yasuda, C. Qian, Z. Qiu, M. Nagamine, H. Ueda, T. Kitaguchi. Customizable OpenGUS immunoassay: A homogeneous detection system using β -glucuronidase switch and label-free antibody. Biosens Bioelectron. 267, 116796 (2025)

Related product

DS540	BCA Protein Assay Kit
DS550	BCA Protein Assay Kit with BSA standard
HBL-C-1	Cry j 1, Cedar Pollen Allergen, Purified <Purified cedar pollen antigen Cry j 1>
HBL-Ab-1-013	Anti-Cry j1, Mouse-Mono(013) <Anti-Cry j1 monoclonal antibody 013>
HBL-Ab-1-053	Anti-Cry j1, Mouse-Mono(053) <Anti-Cry j1 monoclonal antibody 053>

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