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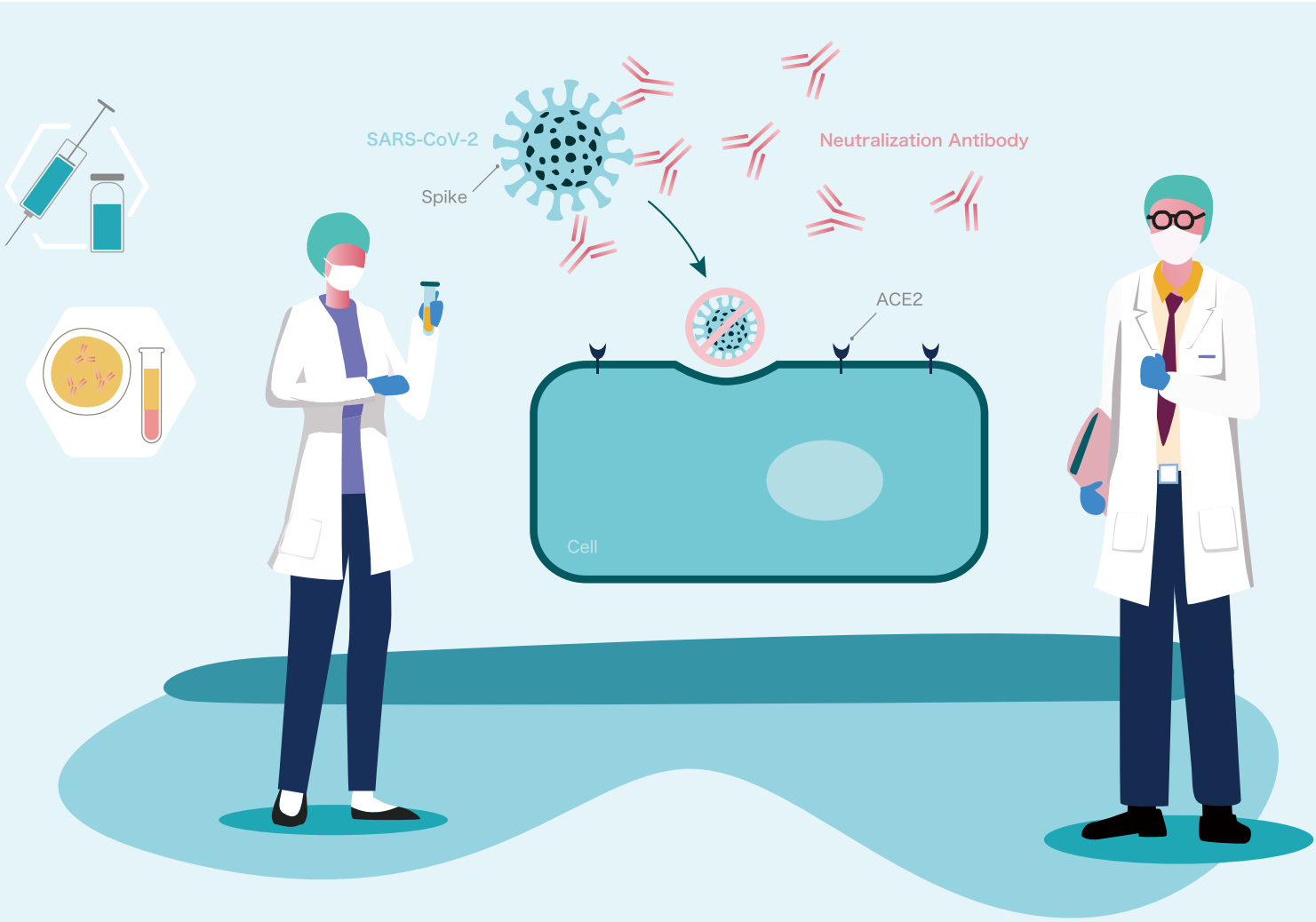
MBL
A JSR Life Sciences Company

Research reagents for severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2)

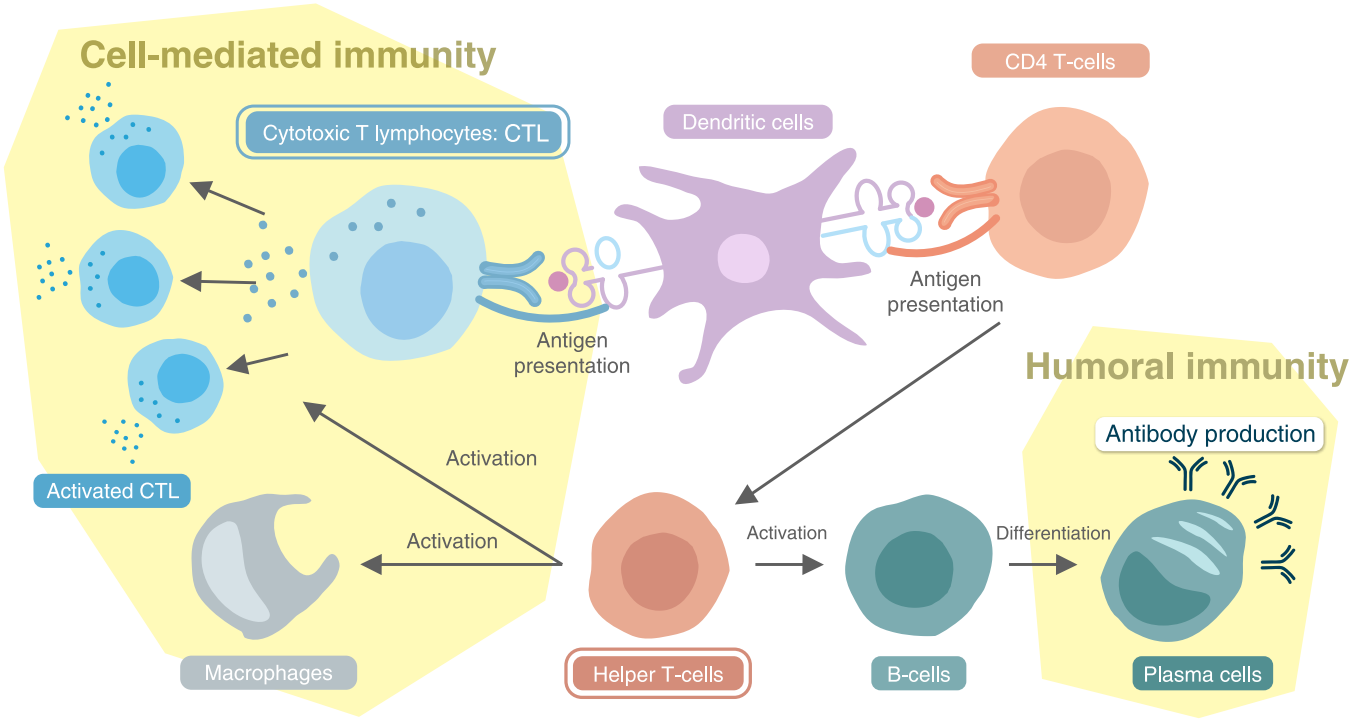
The neutralizing antibody assay kit and reagents for T-cell research

- To perform research on human immunological defence against the novel coronavirus
- For evaluation of induced neutralizing antibodies and T-cell immunity for development of vaccines
- For selection of donors with proper neutralizing antibody in plasma therapy research



The global spread of the novel coronavirus infection has created an urgent need for the development of effective treatments and vaccines. MBL sells reagents for research that are used to evaluate the effectiveness of **“inducing neutralizing antibodies”** and **“inducing T-cell immunity”** during product development.

Acquired immunity (humoral and cell-mediated immunity)



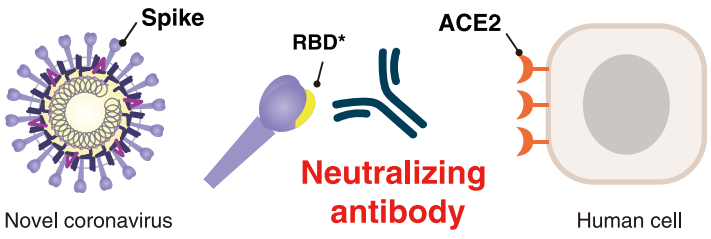
Acquired immunity is a mechanism whereby the immune system specifically identifies and memorizes the pathogen, enabling efficient elimination when the same pathogen is encountered again. This is also known as the adaptive immune system. The immunocompetent cells activated through this mechanism are primarily lymphocytes, such as T cells (cytotoxic T lymphocytes [CTL], helper T cells, etc.) and B cells.

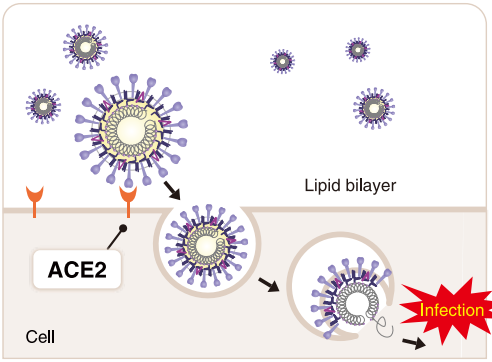
Acquired immunity is further divided into cell-mediated immunity and humoral immunity, depending on the type of helper T cells and their mechanism of action.

Cell-mediated immunity is a localized immune response where CTLs and macrophages directly attack cells. CTLs are activated by cytokines produced by helper T cells that recognize antigens presented on dendritic cells. The CTLs then secrete small molecules to attack and eliminate abnormal cells infected with the pathogen. Some CTLs become memory T cells that are retained in the host memory with cytotoxic activity towards foreign bodies.

In contrast, humoral immunity is an immune response primarily involving B cells and antibodies. B cells differentiate into plasma cells activated by cytokines produced by helper T cells; the B cells then produce large quantities of antibodies that circulate in the body fluids to spread throughout the body. Some of the stimulated B cells become memory B cells that memorize antigen information, enabling these cells to respond much more rapidly to reinfection and to produce vast quantities of antibodies with higher affinity for the antigen.

What are neutralizing antibodies?





*RBD: Receptor Binding Domain (The binding domain of ACE2 on the spike is called RBD)

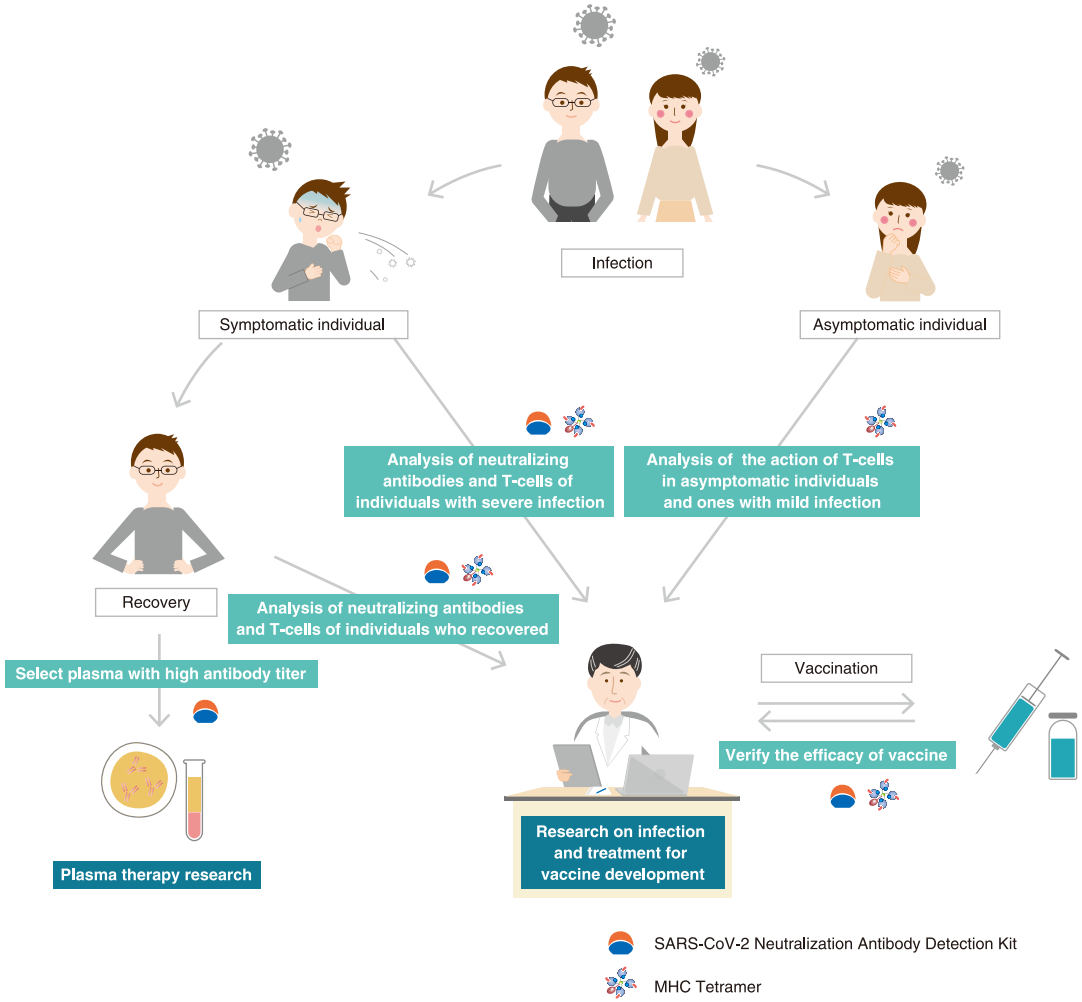
The novel coronavirus infects human cells via the spikes on its surface. Its receptor on human cells is ACE2. To reach the “immunized” state during the recovery from infection or post vaccination, the antibody against the spikes must block the binding of the virus to ACE2. These antibodies are called neutralizing antibodies.

Reagents that detect antibody to SARS-CoV-2 are unable to evaluate neutralization activity. Therefore, separate techniques and reagents that can measure the neutralizing antibodies are required to ascertain whether an infected or vaccinated individual has been “immunized.”

The novel coronavirus and T-cell immunity

With the advancement of research on the novel coronavirus, some reports suggest that the T-cells retain memory of previous coronavirus infections and thus, can potentially elicit an immune response against the novel coronavirus infection (Grifoni A *et al.*, Cell, 181, 1489-1501; Le Bert N *et al.*, Nature 584, 457-462). Therefore, analyzing T-cell activation as well as neutralizing antibodies may lead to better understanding of the effect of the vaccine and immune function against the novel coronavirus.

Novel coronavirus infection and related research



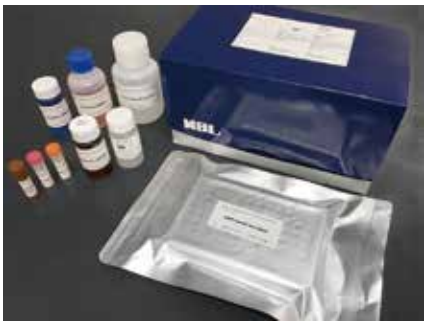
AZ point

What is plasma therapy?

The blood of convalescent patients contains immunoglobulins (antibodies) that neutralize the virus. Administration of the plasma of convalescent patient or immunoglobulin is expected to decrease the severity of the disease. In fact, plasma therapy has been used in several countries to treat patients with Middle East respiratory syndrome (MERS), severe acute respiratory syndrome (SARS), and Ebola haemorrhagic fever.

MBL sells two types of products for SARS-CoV-2 research. “SARS-CoV-2 Neutralization Antibody Detection Kit” can be used to evaluate the neutralization ability in patient samples, and “MHC Tetramer” can be used to detect the activated antigen-specific T cells after infection.

SARS-CoV-2 Neutralization Antibody Detection Kit



This kit is used to evaluate the ability of the antibody to neutralize SARS-CoV-2 in samples through measuring the inhibition of binding between the spikes and the human receptor ACE2.
RBD, the binding domain of ACE2, is used as the spike antigen in the reagent.
Keio University, our collaborative research partner, has confirmed that the results obtained using this kit correlate well with virus neutralization test* results.
A &BSL3 facility is required to perform the virus neutralization tests, and it takes several days to obtain the results. This kit does not use the virus, and it enables evaluation of the neutralization ability in a large number of samples rapidly and easily.

*virus neutralization test: Implemented in accordance with protocol established by the National Institute of Infectious Diseases
&BSL3: Bio Safety Level 3

Kit components

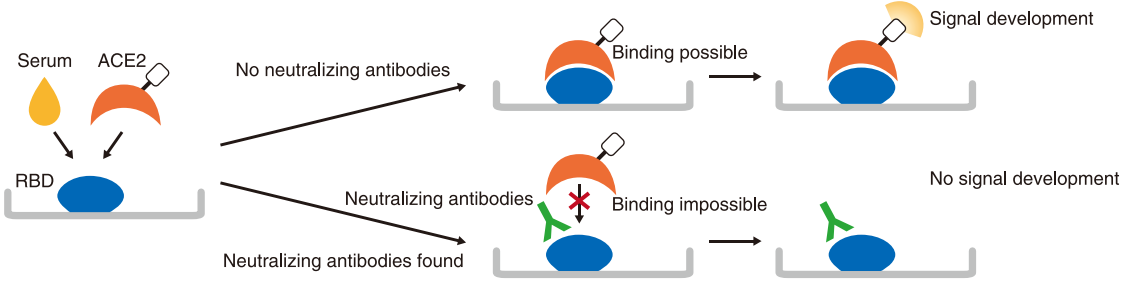
Name	Materials	Quantity
RBD coated microplate	Microwell strips coated with spike RBD recombinant protein	8-well × 12 strips
Positive control	Human derived monoclonal antibody (IgG)	50 µL × 1 vial
Reaction buffer	Reaction buffer (Ready-to-use)	50 mL × 1 vial
Wash concentrate (10x)	Buffer for washing microwells (10x)	100 mL × 1 bottle
ACE2 concentrate	His tagged human ACE2 protein (301x)	50 µL × 1 vial
Conjugate diluent	Buffer for diluting HRP conjugated antibody (Ready-to-use)	20 mL × 1 bottle
HRP conjugated antibody	HRP conjugated anti-His-tag monoclonal antibody (401x)	50 µL × 1 vial
Substrate solution	TMB/H_2O_2 solution (Ready-to-use)	20 mL × 1 bottle
Stop solution	0.5N H_2SO_4 solution (Ready-to-use)	20 mL × 1 bottle
Plate seals	Plate seals	3 pieces

Measurement principles

This kit measures the inhibitory activity of antibodies in a sample on the binding between the spike and ACE2, using the competition method.
A sample is added to RBD coated on the kit reaction plate and reacted. After washing the wells, ACE2 fused with His-tag is reacted. The ACE2-His bound to RBD is quantitatively detected using HRP-labeled anti-His-tag antibodies.

When neutralizing antibodies are absent
ACE2-His binds to RBD. ACE2 bound to RBD is detected using the HRP-labeled anti-His-tag antibodies.

When neutralizing antibodies are present
Binding of ACE2-His is inhibited by the binding of SARS-CoV-2 antibody to RBD, which reduces the amount of signal development.
Alternatively, the greater the inhibitory activity in the sample, the weaker is the signal development.

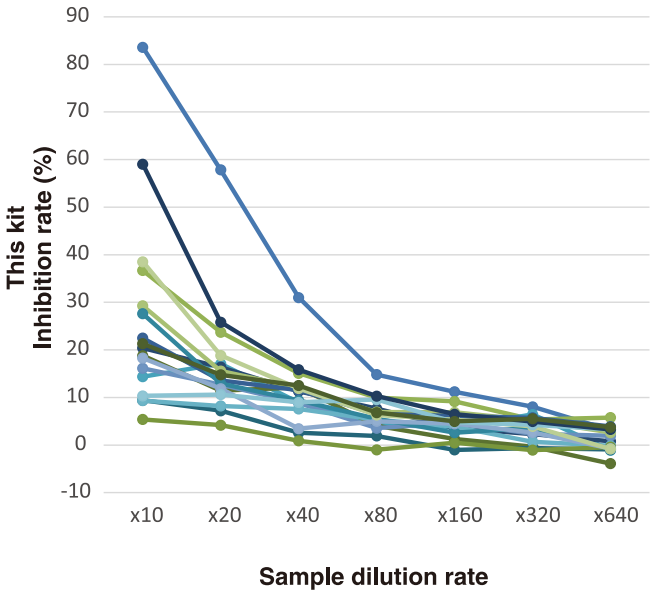


Measurement example

We prepared dilution series of serum samples obtained from 17 patients infected with the novel coronavirus. The absorbance of the sample dilution series and a blank (without patient sample) was measured to estimate the inhibition rate.

$$\text{Inhibition rate (\%)} = \frac{\text{Blank absorbance} - \text{sample absorbance}}{\text{Blank absorbance}}$$

The results showed a sample concentration-dependent inhibition of spike-ACE2 binding, thereby confirming that this kit can be used to measure inhibition activity of the spike-ACE2 binding in patient samples.

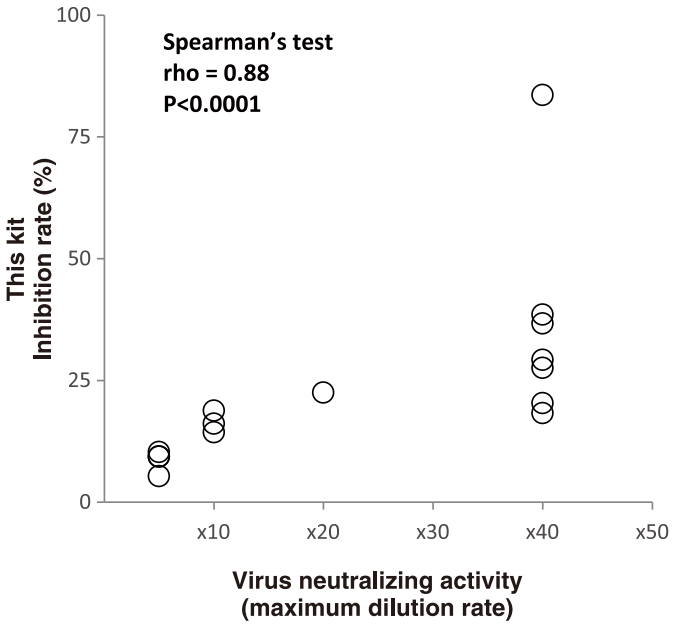


Correlation in virus neutralization test

We assessed the serum samples obtained from 15 patients infected with the novel coronavirus (diluted 10 times) using this kit and evaluated the inhibition rate using the blank measurement values.
We then compared the results of the spike-ACE2 inhibition rate and observed a significant correlation.
(Spearman's test; rho = 0.88, p<0.0001)

Thus, we confirm that this kit can be used to easily evaluate the virus neutralization activity in patient samples.

#Virus neutralization test
The neutralization test was performed according to the protocol established by the National Institute of Infectious Diseases using the Vero-E6/TMPRSS cells as virus-infected cells.
The neutralization titer was evaluated based on the maximum dilution rate at which the neutralization was confirmed.



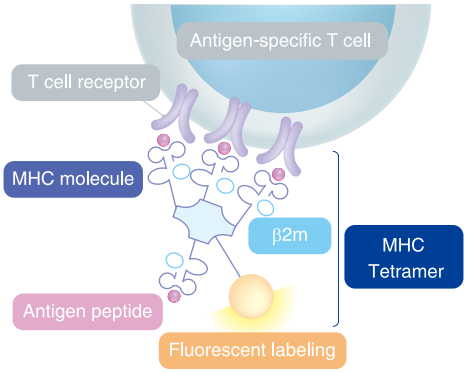
Product list

Code No.	Product name	Size
5360	SARS-CoV-2 Neutralization Antibody Detection Kit	96 wells

Reagents for T-cell research

What is MHC Tetramer?

MHC Tetramer is a reagent of tetramized peptide-MHC complex in order to enhance the binding to T cell receptors. It can be used for experiments to detect the antigen-specific T-cells. It can directly detect SARS-CoV-2 antigen-specific T-cells, unlike the indirect detection methods such as cytokine assay. Moreover, it can also isolate the cells with sorting. This enables detailed functional and phenotypical analysis of T-cells.



Application in novel coronavirus research

■ **Analysis of the antigen-specific T-cells induced post infection**
MHC Tetramer can be used to identify the types of induced T-cell epitopes.

■ **Identification of peptides with high level of immunogenicity for vaccine development**
MHC Tetramer can identify induced T-cell epitopes, which can later be deployed for vaccine development.

■ **Confirm and evaluate the induction of immune response in T-cells upon vaccination**
MHC Tetramer can confirm whether the target T-cell is induced and exhibits sustained response upon vaccination.

SARS-CoV-2 MHC Tetramer/MHC Monomer

It has been reported that the presence of antigen-specific T-cells in human samples has been confirmed using MHC Tetramer presenting HLA-A*02:01 restricted spike peptides. MBL sells these MHC Tetramers and biotinylated MHC Monomers.

< References >

1. Zhang Y *et al.*, bioRxiv. doi: 10.1101/2020.05.24.111823 (2020)
2. Shomuradova AS, *et al.*, bioRxiv. doi: 10.1101/2020.05.20.20107813 version 2 (2020)
3. Chour W *et al.*, bioRxiv. doi: 10.1101/2020.05.04.20085779 (2020)
4. Wang B *et al.*, Blood. 104, 200–206 (2004) PMID: 15016646

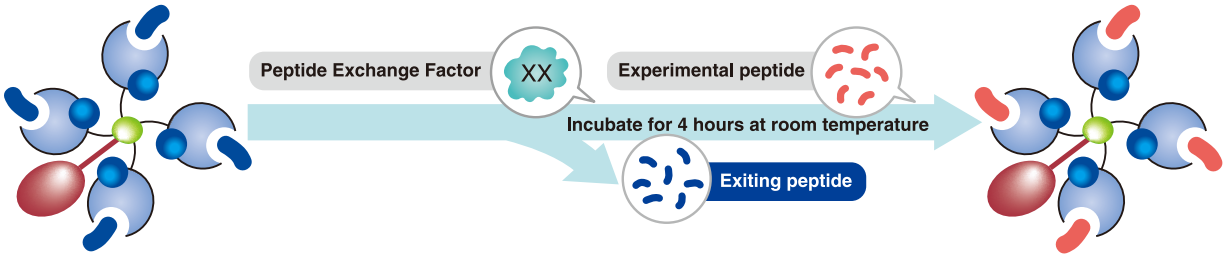
SARS-CoV-2 epitope candidate peptides

We possess a large repertoire of peptides confirmed to exhibit binding ability towards the MHC allele.
Target antigens: S, N, E, M, surface protein, ORF1ab, ORF3a, ORF7a

These peptides can be used in experiments to induce antigen-specific T cells, and to easily prepare MHC Tetramers using the QuickSwitch™ Custom Tetramer Kit.

QuickSwitch™ Custom Tetramer Kit

This kit can be used to prepare the MHC Tetramer reagents using our unique peptide exchange reaction technology. Up to ten MHC Tetramers can be generated just by mixing the peptides of interest. The MHC Tetramers which are generated by the kit and SARS-CoV-2 peptides can be used to identify the types of induced T-cell epitopes after infection.



Reagents for T-cell research – Product list

SARS-CoV-2 epitope-loaded MHC Tetramer/Biotinylated MHC Monomer　All product size: 50 tests

Antigen	MHC	Sequence	PE label	APC label	BV421 label	Biotinylated MHC Monomer
SARS-CoV-2 spike glycoprotein	HLA-A*02:01	RLNEVAKNL	TB-0174-1	TB-0174-2	TB-0174-4	TB-0174-M
SARS-CoV-2 spike glycoprotein	HLA-A*02:01	KLPDDFTGCV	TB-0175-1	TB-0175-2	TB-0175-4	TB-0175-M
SARS-CoV-2 spike glycoprotein	HLA-A*02:01	YLQPRTFLL	TB-0176-1	TB-0176-2	TB-0176-4	TB-0176-M
SARS-CoV-2 spike glycoprotein	HLA-A*02:01	RLQSLQTYV	TB-0177-1	TB-0177-2	TB-0177-4	TB-0177-M

SARS-CoV-2 epitope candidate peptides　All product size: 1 mg

Code No.	Sequence	Region	Alleles with confirmed binding ability
SPC001	EAFEKMVSLLSVLLS	ORF1ab (3906-3920)	HLA-DR1, DR4, DR15
SPC002	EEIAIILASFSASTS	ORF1ab (471-485)	HLA-DR1, DR4, DR15
SPC003	ESPFVMMSAPPAQYE	ORF1ab (1801-1815)	HLA-DR1, DR4, DR15
SPC004	FYVYSRVKNLNSSRV	E (56-70)	HLA-DR1, DR4, DR15
SPC005	LEASFNYLKSPNFSK	ORF1ab (2211-2225)	HLA-DR1, DR4, DR15
SPC006	LLLLDRLNQLESKMS	N (221-235)	HLA-DR1, DR15
SPC007	LSYYKLGASQRVAGD	M (176-190)	HLA-DR1, DR4, DR15
SPC008	MDLFMRIFTIGTVTL	ORF3a (1-15)	HLA-DR1, DR4, DR15
SPC009	MWLSYFIASFRLFAR	M (91-105)	HLA-DR1, DR4, DR15
SPC010	RAMPNMLRIMASLVL	ORF1ab (5016-5030)	HLA-DR1, DR4, DR15
SPC011	SEFSSLPSYAAFATA	ORF1ab (3946-3960)	HLA-DR1, DR4, DR15
SPC012	TRFQTLLALHRSYLT	S (236-250)	HLA-DR1, DR4, DR15
SPC013	WPQIAQFAPSASAFF	N (301-315)	HLA-DR1, DR4, DR15
SPC014	YFTSDYYQLYSTQLS	ORF3a (206-220)	HLA-DR1, DR4
SPC015	FLAHIQWMV	ORF1ab (3122-3130)	HLA-A2
SPC016	FLLNKEMYL	ORF1ab (3183-3191)	HLA-A2
SPC017	LLLDDFVEII	ORF1ab (6749-6758)	HLA-A2
SPC018	LLYDANYFL	ORF3a (139-147)	HLA-A2, A24
SPC019	SMWALIISV	ORF1ab (3732-3740)	HLA-A2
SPC020	TLMNVLTLV	ORF1ab (3710-3718)	HLA-A2
SPC021	YLDAYNMMI	ORF1ab (6419-6427)	HLA-A2
SPC022	YLNTLTLAV	ORF1ab (6851-6859)	HLA-A2
SPC023	YLYALVYFL	ORF3a (107-115)	HLA-A2
SPC024	ASMPTTIAK	ORF1ab (2192-2200)	HLA-A3, A11
SPC025	KSAGFPFNK	ORF1ab (4892-4900)	HLA-A3, A11
SPC026	KTFPPTEPK	N (362-370)	HLA-A3, A11
SPC027	STFNVPMEK	ORF1ab (2600-2608)	HLA-A3, A11
SPC028	TTIKPVTYK	ORF1ab (1875-1883)	HLA-A3, A11
SPC029	DYVYNPFMI	ORF1ab (6159-6167)	HLA-A24
SPC030	FYGGWHNML	ORF1ab (4986-4994)	HLA-A24
SPC031	NYLKRRVVF	ORF1ab (3159-3167)	HLA-A24
SPC032	VNFNFNGL	S (539-546)	H-2K^b
SPC033	MAYRFNGI	S (902-909)	H-2K^b
SPC034	INITRFQTL	S (233-241)	H-2K^b
SPC035	VVLSFELL	S (511-518)	H-2K^b
SPC036	SIVRFPNI	S (325-332)	H-2K^b
SPC037	GNYNYLYRL	S (447-455)	H-2K^b
SPC038	VVFLHVTYV	S (1060-1068)	H-2K^b, HLA-A2
SPC039	SIIAYTMSL	S (691-699)	H-2K^b, HLA-A2
SPC040	KLPDDFTGCV	S (424-433)	HLA-A2
SPC041	VTQLYLGGM	ORF1ab (5385-5393)	H-2K^b
SPC042	ISDEFSSNV	ORF1ab (5583-5591)	HLA-A2
SPC043	ITGLYPTL	ORF1ab (5573-5580)	H-2K^b
SPC044	AAYYVGYL	S (263-270)	H-2K^b
SPC045	YNYLYRLF	S (449-456)	H-2K^b
SPC046	ADYSVLYNSASFSTF	S (363-377)	HLA-DR1, DR4, DR15
SPC047	IWLGFIAGLIAIVMV	SR (1216-1230)	HLA-DR1, DR4
SPC048	LAFVVFLLVTLAILT	E (21-35)	HLA-DR1
SPC049	MKIILFLALITLATC	ORF7a (1-15)	HLA-DR1, DR4
SPC050	NRNRFLYIIKLIFLW	M (41-55)	HLA-DR1, DR4, DR15
SPC051	RVVVLSFELLHAPAT	S (509-523)	HLA-DR1, DR4, DR15
SPC052	TQDLFLPFFSNVTWF	S (51-65)	HLA-DR1, DR4, DR15

Abbreviation
ORF1ab: ORF1ab polyprotein, E: envelope protein, N: nucleocapsid phosphoprotein, M: membrane glycoprotein, S: spike glycoprotein, ORF3a: ORF3a protein, ORF7a: ORF7a protein, SR: surface glycoprotein
HLA-A2: HLA-A*02:01, HLA-A3: HLA-A*03:01, HLA-A11: HLA-A*11:01, HLA-A24: HLA-A*24:02, HLA-DR1: HLA-DRB1*01:01, HLA-DR4: HLA-DRB1*04:01, HLA-DR15: HLA-DRB1*15:01

QuickSwitch™ Custom Tetramer Kit

Product name	Code No.		
	PE label	APC label	BV421 label
QuickSwitch™ Quant HLA-A*02:01 Tetramer Kit	TB-7300-K1	TB-7300-K2	TB-7300-K4
QuickSwitch™ Quant HLA-A*11:01 Tetramer Kit	TB-7304-K1	TB-7304-K2	TB-7304-K4
QuickSwitch™ Quant HLA-A*24:02 Tetramer Kit	TB-7302-K1	—	TB-7302-K4
QuickSwitch™ Quant H-2Kb Tetramer Kit	TB-7400-K1	TB-7400-K2	TB-7400-K4

The tetramer reagent can be used to prepare 10 peptide sequences per kit according to the manufacturer's protocol. There are approximately 2.5 μg tetramer molecules corresponding to each peptide sequence. Please consider the amount to be used when assessing the T-cells in biological samples, such as peripheral blood, for each peptide sequence.

For Research Use Only. Not for use in diagnostic procedures.

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