Cat. # MK100

For Research Use

TaKaRa

Peptide Coating Kit

Product Manual

v201901Da

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I. Description

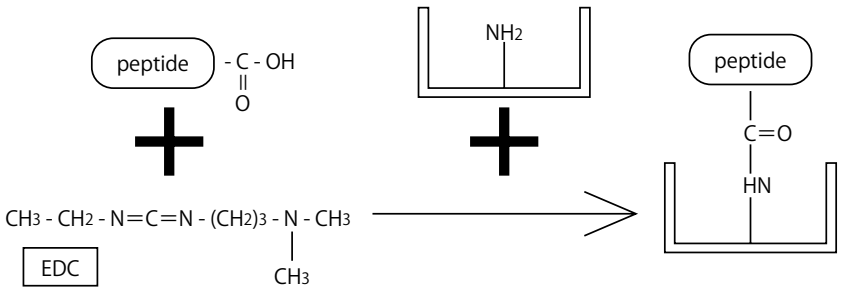
The Peptide Coating Kit is a reagent kit that allows to perform the coating of low molecular weight protein or synthetic peptide efficiently, that are difficult to adsorb on plate spontaneously.

II. Intended Use

- Detection of the antibody (by ELISA) against peptides, sugar chains, medicines and nucleic acids, etc.
- Measurement of the peptide antigens. (By competitive ELISA)
- Analysis of the biologically active peptides and sugar chains. (Cell adhesion test, etc.)

III Principle

Free carboxylic acid group (-COOH) of the peptides are activated by the coupling reagent (EDC) and allowed to couple to the amino group (-NH_2) of the bottom surface of the microplate wells. Coating of sugar chains, medicines and nucleic acids which have free carboxylic acid group are also available.



IV. Contents (for 96 well x 5)

(1) Reaction plate	96 well plate (8 wells x 12 strips) x 5
(2) Coupling reagent	50 mg (for 5 ml)
(3) Reaction buffer	50 ml
(4) Blocking buffer (for ELISA)	50 ml x 2

V. Procedure

1) Preparation of the sample solution.
Dissolve the target peptide* in a concentration of 4 μg/ml with (3) Reaction buffer and aliquot 50 μl of the solution into each well of (1) Reaction plate.

* Peptide cross-linking reaction may be obstructed by buffer components (Tris, glycine, etc.) that have an amino group or carboxyl group, therefore the absence of such chemicals in sample protein or peptide should be confirmed before using the kit.

2) Coupling
<For preparation of 5 plates>
Dissolve the contents of (2) Coupling reagent by adding 5 ml distilled water, then quickly add 10 μl of the reagent into each well of the plate prepared at step 1). After mix well, incubate the Reaction plate for 2 hours at room temperature.

<For preparation of 1 plate>
After aliquot 10 mg of (2) Coupling reagent into a microcentrifuge tube, dissolve it with 1 ml distilled water. Add quickly 10 μl of the reagent into each well of the plate prepared at step 1). After mix well, incubate the Reaction plate for 2 hours at room temperature.

Note
1. In case of preservation of (2) Coupling reagent after using its part, certainly keep it dry at 4℃ (seal with desiccant).
2. Requirement of (2) Coupling reagent should be taken out and be dissolved in distilled water immediately before use.

3) Remove the solution in the wells by pouring out or by suction, and vigorously tap onto paper towel. Wash each well 3 times with distilled water. In each washing step, empty out the plate and vigorously tap onto paper towel.

4) Blocking (for the use in ELISA)
In case of using the peptide-coated plate for the detection of anti-peptide antibody, the plate should be blocked by using (4) Blocking buffer to avoid non-specific adsorption of proteins to the bottom of the wells. Aliquot 200 μl of Blocking buffer into each well and incubate for 1 hour at room temperature. Remove the solution of the wells by pouring out or by suction, and vigorously tap onto paper towel. Wash each well 3 times with distilled water.

VI. Storage of the peptide-coated plates

If the peptide-coated plate is stored, do not treat with Blocking buffer the plate (V-4). Prepare the peptide-coated plate as procedure (V1 - V3), remove the moisture by vigorously tapping onto paper towel, and then keep dry in desiccator at 4℃.

VII. Precaution

1) Peptide to be coated should be purified. Contamination proteins, peptides, amino acids, and some buffer solution (Tris, etc.) will disturb the coating of sample peptides.

2) Samples to be coated should have free carboxylic acid group (-COOH).

VIII. Examples of Use

(1) Production of antibodies against hapten antigens and detection of the specific antibodies

Formerly, production of antibodies had to start from the purification of the antigenic material, which required considerable time. Recently, large numbers of amino acid sequences of proteins have been reported, and because automatic peptide synthesis technology has become pervasive, specific antibodies have become quite easily and reliably produced when the amino acid sequence of the target protein is known, by using a synthetic peptide of that region and sensitizing an appropriate laboratory animal. In this case the length of peptide that can be synthesized by an automatic synthesizing apparatus is about 20 - 30 residues, and they are usually treated as hapten antigens. Since antibodies against both the carrier protein (usually BSA or KLH) and peptide are produced when the peptide is bound to the carrier and the animal is sensitized, the problem is to determine the amount among them that are specific antibodies of the target peptide. For this, this kit is useful to detect the antibodies specific to the peptide by ELISA, using a 96 well microplate directly coated with antigenic peptide.

(2) Solid phase adsorption of low water-soluble, low molecular weight peptides

When peptides are synthesized for the purpose of antibody production, it is common to target a hydrophilic region, in which case the peptides are generally water-soluble and can easily be solid-phased using this kit. However, if the peptide contains many hydrophobic amino acids such as Phe or Trp, it is sometimes poorly soluble in an aqueous buffer, and solid-phasing becomes a difficult process. In such a case, low water-soluble peptides can easily be solid-phased to the microplate just by preparing a few reagents.

1. Dissolve low molecular weight proteins or oligopeptides containing many hydrophobic amino acids such as Trp, Phe or Leu in dimethylsulfoxide (for spectography) instead of the Reaction buffer provided, at a concentration of 4 μg/ml, and dispense 50 μl to each well of the Reaction plate.
2. Dissolve the Coupling reagent into 5 ml of dimethylsulfoxide, then quickly add 10 μl to each well of the plate and mix well. Allow to stand for 2 hours at room temperature.
3. Remove the reaction mixture from the plate and wash the plate several times with large quantities of purified water.
4. Block the plate with the provided Blocking buffer to prevent non-specific adsorption of antibodies or other proteins to the bottom of the plate (incubate for 1 hour at 37℃).

(3) Detection of peptide-specific antibodies by ELISA

1. Collect blood from a rabbit immunized with the target peptide, prepare the plasma from the blood, dilute the plasma appropriately with PBS containing 1% BSA, and dispense it in the plate.
2. Detect the anti-peptide antibody by sequentially reacting the enzyme-labeled (peroxidase) secondary antibody using peroxidase-labeled anti-rabbit Ig and substrate (ABTS).

Experimental Example

The rabbit serum was produced by using the low water-soluble peptide LFFLVWWDQFG (A portion of Papillomavirus E5 protein) as an antigen. The serum was sequentially diluted 10 - fold and reacted in a plate with the antigen coating. The result shows strong antibody reaction to the low water-soluble peptide . The same reaction was conducted in a plate coated with only Lys as a control, and only slight rection to antibody was observed. (Figure 1)

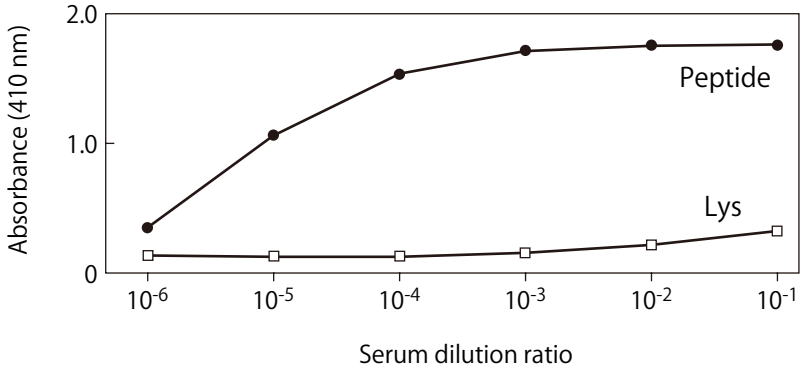


Figure 1. ELISA by specific antibody in a low water-soluble peptide-coated plate