FT-CV2161

Functionalized Microplate

Functionalized microplates for immobilizing covalently biomolecules for ELISA type assays

Products Description

Name: **Amine Reactive MicroPlates**
Catalog #: CV2161, 5 plates kit
96-well plates – bear **NHS groups**
Blocking solution 100 mL

For the immobilization of macromolecules or haptens containing free amine group.
Also available as C8x12 strips clear plates (CV2211) and white plates (CV223)

Name: **Amine Functionalized MicroPlates**
Name: DX1581, 5 plates kit
96-well plates – bear **Amino groups**
Blocking solution 100 mL

For the immobilization of macromolecules or haptens containing free carboxyl group using the EDC mediated amination. Or other possible immobilization strategies.
Also available as C8x12 strips clear plates (CV2271)

Carboxyl **Carboxyl Functionalized MicroPlates**
Catalog #: DX1591, 5 plates kit
96-well plates – bear **Carboxyl groups**
Blocking solution 100 mL

For the immobilization of macromolecules or haptens containing free amino group using the EDC mediated amination.

Storage: Amine & Carboxyl plates: +4°C (L). Can be stored at room temperature for short term.
Amine-reactive plates: +4°C for < 6 months (at –20°C dessicated for long term).

Features & Benefits:

- Covalent attachment – highly stable bond resists harsh conditions (i.e. harsh washing using high ionic strength solutions)
- Single attachment site – avoids to affect the molecular conformation as occurs in passive coating – preserves other sites involved in interactions for assays.
- Oriented immobilization – the active site can be exposed with a better steric availability for interactions yielding a better recognition and specificity.
- Immobilize small peptides without the use of a carrier as needed in passive coating
- Low non-specific binding: i.e. avoids to detect anti carrier abs
- Standard microplate format – compatible with most microplate equipment (washers, readers, automats)
- Standard format Micro-plates with flat bottom wells ()
- Provided with Blocking solution ()

Applications:

- Coating of any molecules: antigens, antibodies, lipids, polysaccharides, nucleic acids.
- Improve assays performances: sensitivity, specificity

Our *Functionalized Microplates* provide a variety of immobilization solutions for quite any molecules.

Plate	crosslinker	Target group on molecule to immobilize and example	
Carboxyl	EDC (&NHS)	NH2 : aminated molecule	Protocol a1
Amine	EDC (&NHS)	COOH : carboxylated molecule	Protocol b1
“	none (direct)	NHS : Succinimidyl activated Molecule	Protocol b2
“	NHS-NHS (DSS)	NH2 : aminated molecule	Protocol b3
“	Glutaraldehyde	NH2 : aminated molecule	Protocol b4
“	NHS- MAL (SMCC)	SH : e.g. SH(Cys)-peptide	Protocol b5
NHS	none (direct)	NH2 : aminated molecule	Protocol c1

These plates are designed to increase the coating density over passive methods, especially for large proteins, because one single covalent bond is sufficient for stable immobilization. They also are expected to improve the orientation of the

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molecule on the plate for better availability for interactions in assays, improving sensitivity and specificity in downstream assays.

Once functionalized by a peptide/protein or hapten, the plates can be used for a variety of ELISA applications: determine anti-peptide antibody levels, affinity measurements by competition Elisa, assays with adhesion molecules, enzyme - substrate studies, ligand - receptor interactions…

Directions for use: Coupling proteins to plates **CV2161** (direct)
Coupling proteins to plates **DX1581** (EDC method, NHS method, Glutaraldehyde method)

The following protocols make covalent immobilisation of molecules.

Directions for use: Coupling proteins/peptides to Amine reactive Microplates (CV2161)(r)

Aminated biomolecules can be directly coupled to the Amine reactive plate #CV2161. This protocol is designed for proteins, but can also apply for peptides and AminoAlly nucleic acid.

a1● Coupling by EDC method of proteins or peptides via their COOH group, to Amine reactive Microplates CV2161 (0):

Caution: protein samples that contain amine solutes (i.e. Tris buffer) and other substance interfering should be desalted. Allow the plate to reach room temperature.

- Dissolve the protein to be coupled in PBS (Phosphate Buffered Saline) at a concentration of 5 to 20µg/ml.
- Dispense 100µl of the above protein solution to each well.
- Incubate the plate for 30-60min at room temperature.
- Wash the plate twice with the buffer used for the coupling.
- Dispense 150µl of the provided blocking solution (suits most applications. This optional step is typically needed for immunoassays. Other blocking solutions may be used depending on your application.)

Coupled plates uses: Further steps to use the plate in immunoassays typically include:
.100µl/well of primary antibody diluted in PBS 0.1% Tween20 incubated for 30-60min at +37°C.
.washing steps (3x) with PBST (or TBST for AP assays).
.100µl/well of secondary antibody diluted in TBST. Incubate for 1H at 37°C. Wash the plate.
.(for enzyme assays): 100µl of substrate (chromogenic/fluorigenic/Luminogenic)

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Directions for use: Coupling proteins/peptides to Amine functionalized Microplates (DX1581)

Amine functionalized plates can be coupled to the carboxyl group (COOH) of any molecule using the carbodiimide method (EDC) [protocol b1], and more directly molecules that have been activated by N-hydroxysuccinimide (NHS) [protocol b2]. They also can immobilize amines of any biomolecules using a bifunctional crosslinker (DSS type) [protocol b3]. The Amine plate can alternatively be activated by glutaraldehyde to form amide bond with NH2-bearing molecules [protocol b4]. Finally SH-bearing molecules (i.e. Cys-terminated synthetized peptides) can be immobilized on Amine plates using a heterobifunctional crosslinker (SMCC type) [protocol b5].

b1● Coupling by EDC method of proteins or peptides via their COOH group, to Amine functionalized Microplates DX1581(r):

This protocol applies to proteins and peptides (Glu, Asp residues; terminal COOH), but can adapted to any carboxylated hapten like Biotin (r).

▪ Dilute the protein or peptide and SulfoNHS in 50 mM phosphate buffer pH 7-8 (or PBS):

Typically to 1.5µg/ml of a small peptide in phosphate buffer, add 0.18mg SulfoNHS /ml. Then make 3 serial dilutions.
Usual concentration are 0.5 - 5µg for antibodies and 1-3µM (~0.5-2µg/ml) for peptides.
A calibration of this protein/NHS solution by serial dilutions is recommended to optimize the conjugation.
.Apolar peptides (or (carboxylated) haptens) car be dissolved in an organic solvent (i.e. up 60% DMSO).

▪ To each microplate #DX1581 well, add 50µl of peptide/NHS solution(s),
and 50µl of water soluble carbodiimide (EDC).
Typically use a 1.23mg EDC/ml . EDC should be in excess

▪ Incubate at room temperature for at least 2 hours.

▪ Wash the plate twice with the provided blocking buffer used for the coupling.

Rem: Due to the large variety of molecules to be coupled with different chemical functionalities, the exact coupling procedure should be determined by the end user.

Coupled plates uses:
.Proteins or peptides, as well DNA, bound to the Amine surface can be stored at 4°C for up to one month.
.Follow your usual protocol for assay with the plate covalently coupled with protein/peptide. The provided blocking solution suits most applications, notably standard ELISA procedures, with immunserum or Mab, secondary Ab and chromogen.

b2● Coupling NHS activated molecules to Amine functionalized Microplates DX1581(r):

N-hydroxysuccinimide (NHS) active esters link immediately to the plate-surface amino groups. Guidelines are given here using NHS-Biotin to prepare biotinylated plates.

▪ Dissolve ~10mg/ml NHS-Biotin in DMSO (water soluble NHS biotin can be dissolved directly in PBS)
Prepare solution in PBS at desired concentration. A calibration experiment can be done from 100µg/ml to 0.1µg/ml.

▪ Incubate for 1H at room temperature 100µl per well of NHS-Biotin (typically 1µg/ml).
note: The plate should be covered to limit border effects.

▪ Wash the plate once with PBS, and saturate using provide blocking agent (several other agents can be used too).

b3● Coupling by the DSS method of proteins to Amine functionalized Microplates DX1581 (r):

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The DSS coupling method consists to make the amino groups of the plate react with the homobifunctional crosslinker DSS, though one of its succinimidyl group. The amines of the plate are so in the secund succinimidyl group of DSS, and able to react with amines of proteins.

A. Activation with DSS

• Prepare a DSS solution at mM. Add 100µL DSS solution to each well of the Amine plates #DX1581.

• Incubate for one hour at room temperature

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Rem: DSS is added in exces to prevent coupling of both DSS ends to the surface.
Brief activation (one hour) with DSS limited hydrolysis of the active NHS-groups.

B. Conjugation

• Empty the wells and add 100µL Protein solution . Cover the wells and incubate overnight at room temperature.
Rem: the protein concentration should be calibrated. Depending on protein MW and nature, the can be in range of 0.1-100µg /ml.

C. blocking

• Empty the wells, add 100µL of provided blocking solution and leave for 15minutes.

• Empty the wells and wash three times with distilled water.

D. Conjugate Incubation

• Empty the wells and add 100µL conjugate solution to the wells.

• Cover the wells, and incubate for two hours at room temperature.

• Empty the wells and wash three times with distilled water.

Store the plate at +4°C. Use the protein covalently coated plate in suitable assay.

b4● Coupling by the Glutaraldehyde method of proteins to Amine functionalized Microplates DX1591[0]:

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For some applications it may be advantageous to activate the Amine surface itself rather than the molecule which is to be bound to the surface. The Amine functionalized plate can be activated by glutaraldehyde, that will replace the secondary amino group with an aldehyde function. The plate can then be coupled to amine groups of a protein or any other molecule bearing amine groups.

A. Activate the Amine plate by glutaraldehyde:

• Add 100µL of glutaraldehyde 1.25% in Phosphate buffer 50mM pH8.2, to each well.
Note: depending on the plate kind (neat PS versus treated PS,…) and quality (brand), one could calibrate the glutaraldehyde concentration, i.e. 5%, 2.5%, 1.25% , and make control with 0% glutaraldehyde. Usually 1.25% of glutaraldehyde is sufficient.

• Incubate overnight at 37°C.

• Empty the wells and wash three times with phosphate buffer.

B. Coupling to activated plate surface

• Add 100µL of the aminated molecule to be coupled, to each well.

Rem: Calibrate the required concentration. This glutaraldehyde method may require a higher concentration than other protocols, 0.1 to 1mg/ml and even much higher for small haptens.

• Incubate 3 hours at 37°C.

• Empty the wells and wash three times with PBS.

b5● Coupling by the MAL/NHS method of SH-bearing peptides to Amine functionalized Microplates DX1581[0]:

The MAL/NHS coupling method is referred as an oriented conjugation method. The amino groups on the plate react with succinimidyl group of a heterobifunctional crosslinker NHS-MAL, hence is converted to other functional group, a maleimide. Then the maleimide group reacts readily and very specifically in mild conditions with free sulfhydryl of the target molecule.
See the technical sheets of SMCC (FT-17412A) and MAL-PEO$_n$-NHS (FT-AL6581; FT-DY6611).
This method is a great approach when a specific coupling site should be targeted, as a native sulfhydryl group that is native or introduced adequately in the molecule to immobilize. This is taken to good account for peptides synthetized with a terminal Cys residue as a handle for engineering. Sulfhydryls can also be generated from other groups by biochemical reactions (i.e. from amines converted by the SATA reagent; from any group by click chemistry), or genetically (introduction of Cys in expressed proteins. Applied in SCAM method).
This method is popular for any molecules containing a free sulfhydryl not involved in the bioactivity, but also oppositively useful to study sulfhydryls that are involved in the bioactivity (block them).

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Directions for use: Coupling proteins/peptides to Carboxyl funct. Microplates (DX1591)(r)

Aminated biomolecules can be coupled to the carboxylated plate # DX1591 via EDC-mediated amidation [protocol c1], similar to protocol b1. Conventional chemistry offers many other conjugations kinds to attach the plate's carboxyl to other target groups of the molecules to be immobilized.

c1● Coupling by EDC method of proteins or peptides via their NH2 groups, to Carboxyl functionalized Microplates DX1591 0:

Caution: protein samples that contain amine solutes (i.e. Tris buffer) and other substance interfering should be desalted. Allow the plate to reach room temperature.

- Dissolve the protein to be coupled in PBS (Phosphate Buffered Saline) at a concentration of 5 to 20µg/ml, with EDC at .
- Dispense 100µl of the above protein solution to each well.

- Prepare a solution of SulfoNHS in 50 mM phosphate buffer pH 7-8 (or PBS): typically at 0.18mg SulfoNHS/ml.
- To each well of microplate #DX1591, add 50µl of the NHS solution(s)
- Prepare a solution of peptides 1.5µM with 1.23mg EDC/ml in 50 mM phosphate buffer pH 7-8 (or PBS).

A preliminar dissolution of peptide with DMSO may be needed.

- Add 50µl of water soluble carbodiimide (EDC) to each well
- Incubate at room temperature for at least 2 hours.
- Wash the plate one time, and dispense 150µl of the provided blocking solution. Incubate for 1H at 37°C
- Wash the plate twice.

FAQ

What is the Amine / NHS / Carboxyl load in wells?
The data is not documented. The plates are quality tested to make a commercial ELISA assay. A definite ligand is immobilized onto the plate using a standard protocol, and the the ligand-plate provides a defined high signal level and low background.

Related / associated products and documents

See BioSciences Innovations catalogue and e-search tool.
Other microplates:
Standard EIA plates #Q89403/Q89414,
FPlyte plates for Fluorescence/Luminescence measurements,
Streptavidin - microplates #L76161, Anti IgG - microplates #47246A/42458A
Crosslinkers, Biotinylation agents, Fluorescent labeling agents:
EDC (carbodiimide) #52005A, Sulfo-NHS #54422A

Ordering information

Catalog size quantities and prices may be found at http://www.interchim.com.

For any information, please ask : Uptima / Interchim; Hotline : +33(0)4 70 03 73 06

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