Solid phase binding assay - Clusterin binding to Very Low-Density Lipoprotein Receptor (VLDLR)

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Protocol status: Working
We use this protocol and it's working

ABSTRACT

This protocol details how to monitor Clusterin binding to the Very Low-Density Lipoprotein Receptor (VLDLR) by Enzyme-linked immunosorbent assays (ELISA) adapted from Leeb et al. (2014).

ATTACHMENTS

Solid phase binding assay_Clusterin binding to VLDLR.docx

MATERIALS

Buffers

- TBS-C: Tris-Buffered Saline pH 7.4, [M] 2 millimolar (mM) $CaCl_2$
- Blocking solution: 2% BSA, 0.05% Tween in TBS-C buffer.
- Quenching solution: [M] 2 Molarity (M) sulfuric acid

Clusterin-α Antibody (B-5) **Santa Cruz Biotechnology Catalog #sc-5289**
RAP Antibody (E-7) **Santa Cruz Biotechnology Catalog #sc-515625**
1-Step™ Ultra TMB-ELISA Substrate Solution **Thermo Fisher Catalog #34028**

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13h

1

Coat the corresponding wells of a 96-well plate (Nunc-Immuno MicroWell 96 well solid plate, MERCK) with 100 µL of TBS-C containing 10 undetermined VLDLR ectodomain Overnight at 4 °C.

Note

The same number of wells should be incubated with TBS-C without VLDLR. These wells will be used as ligand background binding (addition of ligand to wells without immobilized receptor).

2

Wash the plate once with TBS-C.

Note

The washing step should be quick to avoid dilution and detachment of the receptor.

3

Add Blocking solution and incubate the plate for 02:00:00 at Room temperature (25 ºC). The wells without receptor are now coated with BSA.

2h

4

Remove Blocking solution and apply a series of increasing concentrations of ligand diluted in Blocking solution, each in a final volume of 100 µL. Each ligand concentration should be added to one well with immobilized VLDLR and one well coated with BSA (Blocking solution) for ligand background binding. One

well with VLDLR and one well coated with BSA should be incubated without ligand to determine the general plate background signal.

Note

- For Clusterin, a concentration range from 50 nM to 10000 nM is recommended (approximate KD = 80-140 nM).
- Low Density Lipoprotein-Related Protein-Associated Protein 1 (LRPAP1 or RAP) is a molecular chaperone for LDL receptor-related proteins and therefore it can be use as positive control and as a competitor binder. For RAP binding, a concentration range from 1 nM to 60 nM is recommended (approximate KD = 1-2 nM). For competition assays, mix a fixed concentration of the ligand with increasing concentrations of the competitor (RAP).

5 Incubate 01:00:00 at Room temperature (25 °C). 1h

6 Wash the plate three times with Blocking solution.

Note

If testing the effect of pH on ligand binding, wash the wells once with TBS-C Blocking solution (pH 7.4) or a low pH buffer like SA-C Blocking solution ([M] 10 millimolar (mM) Na-acetate pH 5.2, [M] 150 millimolar (mM) NaCl, [M] 3 millimolar (mM) $CaCl_2$, 2% BSA, 0.05% Tween) and incubate with the corresponding buffers for 01:00:00 at Room temperature (25 °C). After the incubation time, wash the plate once with the same buffers.

7 Add the corresponding primary antibodies diluted 1/100 in Blocking solution and incubate 01:00:00 at Room temperature (25 °C). 1h

Note

anti-Clusterin (sc-5289 Santa Cruz Biotechnologies) and anti-RAP (sc-515625 Santa Cruz Biotechnologies) can be used for Clusterin and RAP detection, respectively.

8 Wash the plate three times with TBS-C Blocking solution.

9 Add the corresponding secondary antibody (horseradish peroxidase (HRP) conjugated) diluted 1/10,000 in Blocking solution and incubate 01:00:00 at Room temperature (25 °C). 1h

10 Wash the plate three times with TBS-C Blocking solution.

11 Add 100 µL per well of the HRP substrate 1-Step Ultra TMB ELISA Substrate Solutions (Thermo Fisher Scientific, 34028) to develop the plate and incubate until the desired color develops.

Note

3 and 10 minutes incubation time with the developing solution are normally enough under these conditions to develop RAP and Clusterin signal, respectively.

12 Add 100 µL per well of quenching solution to stop the reaction.

13 Measure absorbance at 450 nm.

Note

First, subtract the background signal of each sample (VLDLR coated well – BSA coated well). Next, subtract plate background from each sample (wells incubated without ligand).