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

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

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#### **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 (рн 7.4 , [м] 2 millimolar (mM) CaCl<sub>2</sub>
- Blocking solution: 2% BSA, 0.05% Tween in TBS-C buffer.
- Quenching solution: [M] 2 Molarity (M) sulfuric acid
- X Clusterin-α Antibody (B-5) Santa Cruz Biotechnology Catalog #sc-5289
- RAP Antibody (E-7) Santa Cruz Biotechnology Catalog #sc-515625
- X 1-Step™ Ultra TMB-ELISA Substrate Solution Thermo Fisher Catalog #34028



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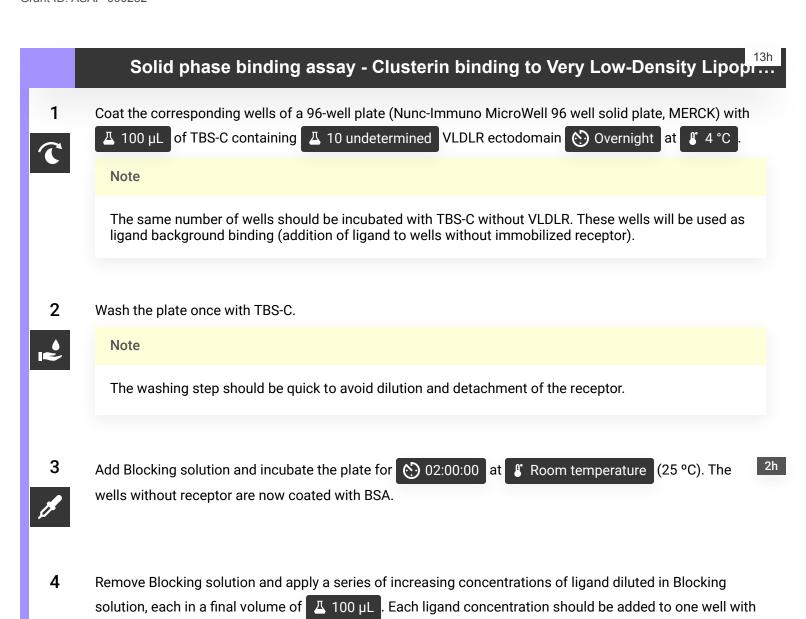
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immobilized VLDLR and one well coated with BSA (Blocking solution) for ligand background binding. One

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).













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 ( IMI 10 millimolar (mM) Na-acetate (PH 5.2 , IMI 150 millimolar (mM) NaCl, IMI 3 millimolar (mM) CaCl<sub>2</sub>, 2% BSA, 0.05% Tween) and incubate with the corresponding buffers for 1:00:00 at 1 Room temperature ( 1:25°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





Room temperature ( \$\mathbb{L}\$ 25 °C ).

### 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.

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 1h





Blocking solution and incubate (5) 01:00:00 at \$\mathbb{S}\$ Room temperature (

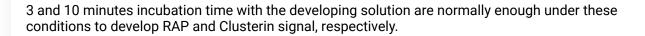
10 Wash the plate three times with TBS-C Blocking solution.





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



12 Add  $\angle$  100  $\mu$ L 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).

