# Sandwich ELISA for IL-8 detection in supernatants

**Stefan Odenbreit, Luisa F. Jiménez-Soto**

## Abstract

The IL-8 ELISA protocol here described was established by Stephan Odenbreit and modified in this form by Luisa F. Jiménez-Soto.

Here are the publications using this protocol:

DOI:10.1371/journal.pone.0035341

DOI:10.1128/IAI.00364-09

DOI:10.1078/1438-4221-00205

and PMID:11886563

**Citation:** Stefan Odenbreit, Luisa F. Jiménez-Soto Sandwich ELISA for IL-8 detection in supernatants. **protocols.io** dx.doi.org/10.17504/protocols.io.gz7bx9n
**Published:** 08 Jun 2017

## Protocol

### Coating of plates

**Step 1.**

- Resuspend 30µl (15 µg) coating antibody (Anti-IL-8 Antibody IL-8 Human 554716 Pharmingen, 0,5µg/µl) in 5 ml Coating buffer (100 mM Na2HPO4 pH 9,6)
- Pipette 50µl of antibody solution in each well of a Nunc MaxiSorp® flat-bottom 96 well plate. Take care to cover the whole surface of each well with the solution. Incubate at 4°C for minimum 8 hours

REAGENTS
Purified Mouse Anti-Human IL-8 554716 by BD Biosciences

### Remove unbound antibody

**Step 2.**

Remove the unbound antibody, washing twice with 200 µl/well washing buffer (PBS 0,05% Tween 20 (v/v))

### Blocking the plate

**Step 3.**

Add 100µl to each well a PBS 10% FBS solution for blocking, for minimum 1 hour at 37°C or 2 hours at room temperature.

REAGENTS

Fetal Bovine Serum 10270106 by Gibco - Thermo Fischer

## Remove blocking solution

**Step 4.**

Remove blocking solution and wash well twice with 200µl Wash buffer per well.

## Prepare standards and samples andadd to plate

**Step 5.**

- Add standard solutions (100µl / well)
- Prepare samples by mixing throughly before loading them onto the plate. (final volume per well: 100 µl).

## Incubate samples

**Step 6.**

Incubate samples for minimum 5 hours at 37°C, or overnight at 4°C. Do not shake.

## Wash to remove unbound molecules

**Step 7.**

Remove thoroughly the samples by washing 6 times with 200 µl wash buffer / well.

## Add the detection antibody

**Step 8.**

Add 100µl/well of a solution of 10µg biotinylated IL-8 antibody (BD Biosciences, Catalog No.554718) in 10 ml wash buffer 10% FBS, and incubate for minimum 45 min at 37°C, or 2 hours at room temperature.

REAGENTS

Biotin Mouse Anti-Human IL-8 554718 by BD Biosciences

## Prepare the Peroxidase (POX)-Avidin complex

**Step 9.**

30 minutes before the incubation of the detection antibody is ready, prepare the POX -Streptavidin complex, by mixing 1,5µl of solution A and 1,5µl of solution B (Vectastain ABC kit, Catalog Number: PK-4000) in 200µl ELISA buffer . Vortex shortly to mix and incubate for 30 min at room temperature.

REAGENTS

VECTASTAIN ABC HRP Kit (Peroxidase, Standard) PK-4000 by Vector Laboratories

## Remove unbound biotinylated antibody

**Step 10.**

Once the incubation of the biotinylated antibody is ready, remove all unbound antibody from plate by

washing 6 times as described before

## Add the POX-Streptavidin solution (detection complex)

**Step 11.**

Prepare the POX-Streptavidin solution by diluting the 200 µl previously prepared in 10 ml of ELISA buffer (50 mM TRIS-HCL pH 7,6). MIX BY INVERSION ONLY. Do not vortex.

**Step 12.**

Add 100µl of the POX-Strep complex solution in each well. Incubate for 45 min at room temperature. Do not vortex the POX-Step complex.

**Step 13.**

Remove the unbound complex, by washing as previously described 6 times. Develop using a TMB substrate (5 ml Solution A + 5 ml Solution B), 100 µl per well, and stop reaction using 50 µl of a 1M $H_2SO_4$. Read in Wavelength described by TMB substrate instructions.

REAGENTS

TMB Substrate Reagent Set 555214 by BD Biosciences