Application Note

Sandwich ELISA for Detection of Collagenase

Protocol for sandwich ELISA suitable for quantification of Collagenase NB 4 Standard Grade, Collagenase NB 5 Sterile Grade or Collagenase NB 6 GMP Grade in solutions. Concentrations as low as 100 ng/ml can be detected with antibodies used in this protocol.

Equipment:

- pH meter
- Multichannel pipette with tips
- Microtitre plate (transparent, 96 wells) with lid
- Microplate reader

Reagents:

- Collagenase standard: Collagenase 4 Standard Grade, Collagenase NB 5 Sterile Grade, or Collagenase NB 6 GMP Grade
- Capture antibody: Anti-Collagenase (*Clostridium histolyticum*), polyclonal antibody from sheep
- Tracer antibody: Anti-Collagenase (*Clostridium histolyticum*), polyclonal antibody from sheep, conjugated with horseradish peroxidase

Buffers and solutions:

- Coating buffer: 50 mM Na_2CO_3, pH 9.6 (adjust with 1 M HCl)
- Phosphate buffered saline (PBS), 1 x, pH 7.4 (adjust with 0.1 M NaOH)
- Wash buffer: PBS with 0.05 % (v/v) Tween 20
- Blocking solution: PBS with 0.1 % bovine serum albumin
- Substrate solution:
  0.2 M Na_2HPO_4 and 0.1 M citric acid in H_2O, pH 5.0 (adjust with 0.1 M NaOH)
  with 0.04 % (w/v) *ortho*-phenylendiamine dihydrochloride
  Attention: *ortho*-phenylendiamine dihydrochloride is light-sensitive!
  Aliquots of the substrate solution can be stored at -20 °C for six month.
- Hydrogen peroxide solution 30 % (w/w) in H_2O
- Stop solution: 2 M H_2SO_4 in H_2O

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Procedure:

1. Dilute the capture antibody in coating buffer to a final solution of 10 µg/ml. Coat the wells by adding 50 µl (500 ng capture antibody) into each well.
2. Seal the microtitre plate with a lid and incubate for at least 16 h at +2 to +8 °C.
3. Invert the plate to empty and gently tap out the residual liquid on a paper towel. Add 300 µl wash buffer per well and incubate for 30 s at room temperature. Wash three times in total. After the last washing step gently tap out residual liquid on a paper towel.
4. Add 300 µl blocking solution per well.
5. Seal the plate and incubate for 30 min at 37 °C.
6. Wash the plate three times (see step 3).
7. For generating a standard curve prepare on ice dilutions of collagenase standard (in triplicate) from ~ 1 ng/ml to 10 µg/ml in fresh buffer which was prepared for washing of the cells. If this buffer is not available, use wash buffer [PBS with 0.05 % (v/v) Tween 20] for dilution of standards. Include buffer without collagenase as blank.
   Dilute on ice samples in the same buffer which was used for dilution of standards.
8. Add 100 µl of above mentioned solutions per well.
9. Seal the plate and incubate for 60 min at 37 °C and 300 rpm (orbital shaker).
10. Wash the plate three times (see step 3).
11. Dilute the tracer antibody in washing buffer to a final solution of 5 µg/ml.
    Add 50 µl (250 ng tracer antibody) per well.
12. Seal the plate and incubate for 60 min at 37°C and 300 rpm (orbital shaker).
13. Wash the plate three times (see step 3).
14. Add 1 µl H_2O_2 per 1 ml substrate solution, mix and add 100 µl per well.
15. Seal the plate and incubate for 20-30 min at room temperature in the dark.
16. Stop reaction by adding 50 µl stop solution per well.
17. Measure the optical density at 492 nm with a microplate reader within 30 min after adding stop solution. Plot standard curve and use it to quantify the collagenase in the buffer used for washing of the cells.

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Order Information

The following products are available from SERVA Electrophoresis:

Product	SERVA Cat. No.
Anti-Collagenase (*C. histolyticum*), polyclonal antibody from sheep	58050
Anti-Collagenase (*C. histolyticum*), polyclonal antibody from sheep, conjugated with horseradish peroxidase	58051
Bovine serum albumin (albumin bovine fraction V, pH 7.0)	11930
Citric acid*H_2O	38640
Collagenase NB 4 Standard Grade	17454
Collagenase NB 5 Sterile Grade	17459
Collagenase NB 6 GMP Grade	17458
Na_2CO_3 (Sodium carbonate)	30181
Na_2HPO_4*2 H_2O (di-Sodium hydrogen phosphate)	39783
PBS (buffer substance Dulbecco's)	47302
Tween 20	39796

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