## Required materials

- Coating buffer: 0.1 M Sodium Carbonate, pH 9.5 (8.4 g $NaHCO_3$, 3.56 g $Na_2CO_3$, 1 litre de-ionized water).
- Wash-buffer: PBS, 0.05% Tween20.
- Blocking-buffer/Assay diluent: PBS, 1% BSA
- Capture antibody: Purified anti-mouse IgE, IgG1, or IgG2a antibodies.
    - Anti-mouse IgE (Citeq part number, HE.02.01), dissolve in 0.5 ml coating buffer, dilute 20x.
    - Anti-mouse IgG1 (Citeq part number, HE.02.02), dissolve in 0.5 ml coating buffer, dilute 20x.
    - Anti-mouse IgG2a (Citeq part number, HE.02.03), dissolve in 0.5 ml coating buffer, dilute 20x.
- Optional: Reference mouse serum (for relative quantification or as a positive control)
- Recommended: Non-immunized mouse serum (for use as a negative control)
- Biotinylated Der p 1, Der p 2, D. pteronyssinus extract or D. farinae extract (available from Citeq):
    - D. pteronyssinus extract Biotinylated (Citeq part number, 02.01.88), dissolve in 0.5 ml Buffer (recommended PBS), dilute 50x.
    - D. farinae extract biotinylated (Citeq part number, 02.02.88), dissolve in 0.5 ml Buffer (recommended PBS), dilute 50x.
    - Der p 1 biotinylated, (Citeq part number, 02.01.73), dissolve in 0.5 ml Buffer (recommended PBS), dilute 20x.
    - Der p 2 biotinylated, (Citeq part number, 02.01.74), dissolve in 0.5 ml Buffer (recommended PBS), dilute 50x.
- Streptavidin-horseradish peroxidase conjugate.
- TMB substrate solution
- Stop solution: 2.5 M $H_2SO_4$.
- Flatbottom 96 Microwell plates.

## Required equipment

- Microplate reader with software
- Microplate washer (optional)

## Method

1. Coat micro wells with 100 µl per well anti-IgE (or IgG1/ IgG2a) capture antibody diluted in coating buffer. Incubate overnight at 4°C.
2. Aspirate wells and wash 3 times with 300 µl/well wash-buffer.
3. Block plates with 300 µl blocking-buffer. Incubate at room temperature for (at least) 1 hour.
4. Aspirate wells and wash 1 time with 300 µl/well wash-buffer.
5. Dilute serum samples and controls/references in assay diluent. Pipette 100 µl of each in duplointo the wells. Incubate for 2.5 hours at room temperature.
6. Aspirate wells and wash 5 times with 300 µl/well wash-buffer.
7. Add 100 µl/well of diluted biotinylated HDM/Der p 1/Der p 2 to each sample and incubate for 1 hour at room temperature.
8. Aspirate wells and wash 5 times with 300 µl/well wash-buffer.
9. Add 100 µl of diluted Streptavidin-Peroxidase to each well, incubate for 30 minutes at room temperature.
10. Aspirate wells and wash 7 times with 300 µl/well wash-buffer.
11. Add 100 µl of substrate solution to each well.
    Incubate plate at room temperature in the dark until:
    a. blanks are staining or
    b. until the positive control stains clearly.
12. Add 50 µl of stop solution to each well.
13. Read absorbance at 450 nm within 30 minutes of stopping reaction. Use A 650 as a reference filter.