

Control of Homogeneity in Adsorption of IgG on MaxiSorp™ and MediSorp™ Surfaces

The certification is based on adsorption of IgG according to the following test procedure where CV is less than 5% and all well results within ±10% from mean.

1. Add antibody coating mixture consisting of Dako X-903 (diluted 1:5000) and Dako P-128 (diluted 1:40,000). Dilution buffer: 0.05 M Carbonate buffer, pH 9.6, 200 µl per well (F-bottom) or 150 µl per well (U-bottom). Seal the wells with adhesive tape to prevent evaporation.

Carbonate buffer: Na₂CO₃ = 1.59 g/l
 NaHCO₃ = 2.93 g/l
 diluted in ultra high purity water

2. Incubate in dark at room temperature overnight (minimum 16 hours).

3. Wash 3 times (Nunc-Immuno™ Wash) in washing buffer.

Washing buffer: NaCl = 20.20 g/l
 KCl = 0.20 g/l
 Na₂HPO₄, 2 H₂O = 1.15 g/l
 KH₂PO₄ = 0.20 g/l
 Triton X-100 = 0.50 ml/l
 diluted in de-ionised water
 pH = 7.2

4. Add substrate – 200 µl per well (F-bottom) or 150 µl per well (U-bottom).

Substrate: Citrate (C₆H₈O₇, H₂O) = 7.30 g/l
 Na₂HPO₄, 2H₂O = 11.86 g/l
 OPD (1,2 Benzene diamine) = 600 mg/l
 Perhydrole (30% H₂O₂) = 500 µl/l
 diluted in ultra high purity water

5. Stop the substrate reaction after 3.5 - 4 minutes by adding 2N H₂SO₄, 150 µl per F-well or 100 µl per U-well.

6. Read O.D. (Optical Density) values at 490 nm against unconverted substrate in a MicroWell™ Plate Reader. F-wells are read at this single wavelength, whereas U-wells are read at dual wavelengths with 620 nm as a reference. It is necessary to read at dual wavelengths for U-wells in order to eliminate possible light refraction in the bottom curvatures. Therefore, dual wavelength reading is also recommended for all assays using U-wells.

7. Calculate the C.V. (Coefficient of Variation) for the O.D. readings of an 8 x 12 matrix of MicroWells according to following formula:

$$C.V. = S \cdot \frac{100\%}{\bar{X}} = \sqrt{\frac{\sum (X - \bar{X})^2}{N-1}} \cdot \frac{100\%}{\bar{X}}$$

where: S = standard deviation* of O.D. readings,
 \bar{X} = mean of O.D. readings,
 X = individual O.D. readings,
 N = number of readings (wells)

* The expression given is equivalent to:

$$\sqrt{\frac{\sum (X^2) - \frac{1}{N} (\sum X)^2}{N-1}}$$

which is more convenient for calculation.