

Thermo Scientific Nunc FluoroNunc Modules and Plates

– The principle behind Q.C. procedure

Key Words

Thermo Scientific™ Nunc™ FluoroNunc™, Thermo Scientific™ Nunc™ MaxiSorp™, fluorescence detection,

Goal

The goal of this application note is to describe how to obtain reliable results from assays based on fluorescence detection.

In order to obtain reliable results from assays based on fluorescence detection, it is of utmost importance to have a low uniform background fluorescence from the reaction vessel, as well as the highest possible positive signal. The Nunc FluoroNunc products have been developed to fulfil these requirements.

The Nunc FluoroNunc product group consists of plates and modules in transparent, white or black surface types, each type matching the different needs required by the different techniques. Transparent plates are intended for use in applications where the lowest background fluorescence is needed and a “read-through” detection system is used, e.g. in some time resolved fluorescence instruments.

White plates give the highest possible reflection of the fluorescent signal with a low background fluorescence. These plates are superb for most applications where the type of reading used is epifluorescence. Black plates give the lowest possible background fluorescence, minimizing the backscatter light generated by using epifluorescence. For read through instruments black or white plates are available with optically clear bottoms.

Materials

The antibodies were from DAKO (Denmark), and 0.05 M carbonate buffer pH 9.6 was used as coating buffer.

Antibody mixture: Rabbit anti-sheep (1:5000) and peroxidase conjugated rabbit anti-human AFP (1:40.000).

PBS (pH 7.2, 0.5 M) + 0.05% Triton X-100 + 0.2 M NaCl (PBS Wash) was used for washing.



3-(p-HydroxyPhenyl) Propionic Acid (HPPA) 20 mm in 0.1 M Tris-HCl pH 8.5 + 0.036% H₂O₂ was used as fluorescent substrate. Reagents were from Sigma unless otherwise stated.

Test method

The test principle is based on the competition between labeled and unlabeled antibodies for binding sites on the surface. The concentration of antibody is fixed at 4 µg/mL (surface saturation conditions). The concentration of labeled antibody is adjusted in order to detect a reasonable signal after substrate reaction. The same procedure is used for all Nunc FluoroNunc products.

The plates are coated (150 µL antibody mixture/well) overnight at RT, covered and placed in the dark, then washed three times using PBS Wash followed by a substrate reaction, 150 µL/well. The reaction is stopped by adding 50 µL/well 1 N NaOH. The results are read in a fluorescence reader, i.e. Fluoroscan II.

In order to assure compatibility with the existing Nunc MaxiSorp product, we have made a non-fluorescent QC control test similar to the one used for Nunc FluoroNunc MaxiSorp certification. This test is based on the same principle as indicated above except for using peroxidase/OPD as substrate/chromogen (see Thermo Scientific Nunc Bulletin No. 4, Aspects of Nunc MaxiSorp MicroWell Certification, for more details).

Results

Comparison of standard MaxiSorp Plates (Fig. 1) and Nunc FluoroNunc plates (Fig. 2) using the standard MaxiSorp non-fluorescence method. Five plates were tested, and the mean and SD of each plate (96 wells) calculated. As can be seen from Fig. 1, similar results are obtained for the two products, i.e. the surface quality of Nunc FluoroNunc MaxiSorp is comparable to the standard MaxiSorp surface quality for antibody adsorption.

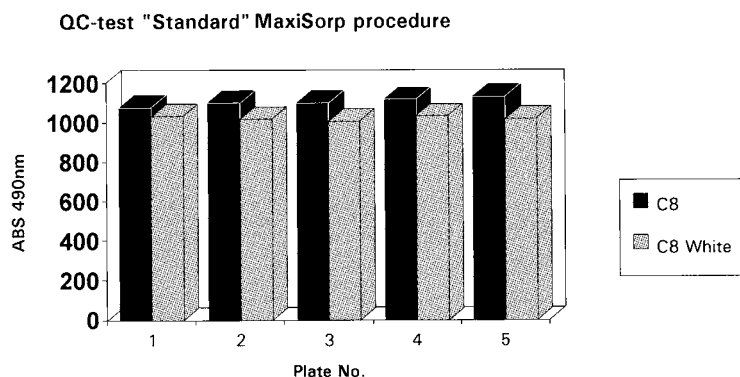


Fig. 1.

QC-test "Standard" Nunc MaxiSorp procedure

Five frames of standard Immuno Module C8, MaxiSorp (Transparent) were compared to five frames of Nunc FluoroNunc Immuno Module, White C8, MaxiSorp. The converted substrate/OPD were, for all plates, transferred to wells of Immuno Plate F96 for reading in Immuno reader NJ-2000. Each bar indicates mean value for each plate.

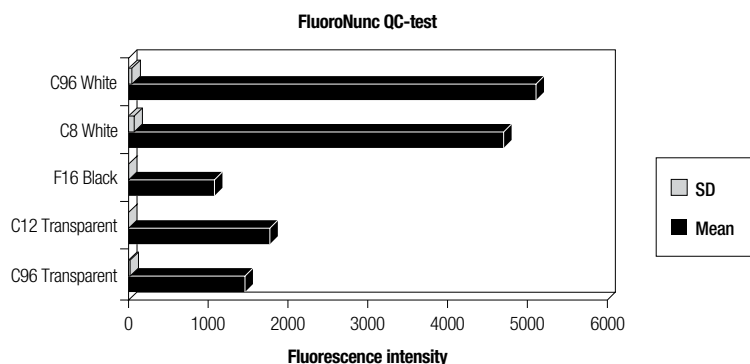


Fig. 2.

QC-test "Fluorescence" procedure

Nunc FluoroNunc Immuno Modules and Plates: White C8, MaxiSorp; White C96, MaxiSorp; Transparent C96, MaxiSorp; Transparent C12, MaxiSorp and Black F16, MaxiSorp, five plates of each, were compared using the Fluorescence procedure. The bars represent the mean and SD values for each product type.

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