Tech Note
No. 12

Thermo Scientific Nunc FluoroNunc Plates and Modules:

A Solid Phase for Fluorescent Immuno Assays

This Tech Note describes the use of white Thermo Scientific Nunc FluoroNunc C96 plates and white C8 modules in a fluorescence immuno assay.

The use of fluorescence techniques in solid phase diagnostic assays is attracting increasing attention due to the higher sensitivity which can potentially be obtained. Sensitivity, however, is not only a question of the right detection system, but is also dependent on the performance of the solid phase used.

The Nunc™ FluoroNunc™ plates and modules have been developed in a transparent version for use in time resolved fluorescence assays where the plate background is minimized. The black version minimizes light scattering, while the white version is used for high sensitivity in traditional fluorescence assays (Tech Note No. 6).

All the FluoroNunc products can be obtained with Thermo Scientific Nunc PolySorp or MaxiSorp surface which provides the binding properties needed to make the most sensitive and reliable assay.

To demonstrate the use of white FluoroNunc plates and modules we have designed a sandwich assay for detection of IgG.

Materials and Method

Solid phase: FluoroNunc Plate, White C96 MaxiSorp™.

Antibodies: Pig anti-rabbit, Rabbit anti-sheep, Pig anti-rabbit alkaline phosphatase conjugate.

Buffers: Carbonate buffer 0.05M pH 9.6. Phosphate buffered saline 0.15M pH 7.2 (PBS). Assay buffer: PBS with 0.05% Tween 20. Wash buffer: PBS with 0.2 M NaCl and 0.05% Triton X100.

Substrate: 0.1 mm 4-methyl-umbelliferylphosphate in diethanolamine buffer pH 9.8.

Miscellaneous: Bovine serum albumin.

Stop solution: 3 M K_2HPO_4.

150 µL carbonate buffer containing 5 µg/mL PaR antibody was added to each well. The wells were sealed with adhesive foil. The plates were coated overnight at room temperature. Then the plates were washed once with demineralized water. Blocking solution (400 µL PBS with 0.5% BSA) was added to all the wells and the plates incubated for 10 minutes at room temperature. The wells were completely aspirated, and a 1:2 dilution series of RaS, 150 µL/well, was performed in the plates (starting in column 2 at a concentration of 420 ng/mL). Assay buffer was added to column 1. The plates were incubated for 2 hours at room temperature, then washed 5 times with wash buffer using Thermo Scientific Nunc Immuno Washer.

Conjugate in assay buffer, 150 µL/well, was added to all wells in dilution 1:500 or 1:5000, and the plates incubated for 2 hours at room temperature. After the plates had been washed 5 times with wash buffer the substrate reaction was initiated by adding 150 µL/well of substrate. The reaction was stopped after 15, 30 or 45 minutes, by adding stop solution.

The fluorescent results were read on a fluorescence reader and the data collected on computer using in house software.

Results

The results of this test are shown in Fig. 1 and graphically in Fig. 2.

As can be seen the best sensitivity was obtained using conjugate in a dilution of 1:500 and a substrate reaction time of 30 minutes. No improvement in sensitivity was observed when the substrate reaction time was increased to 45 minutes. Data analysis, using background (Bg) signal plus 2 SD as cut off value showed that a detection of 20-40 pg/mL RaS antibody could be clearly obtained in this test.

Discussion

The successful performance of solid phase fluorescent technique is dependent on the solid phase used just as much as on a reliable detection system. The result of this test has shown that a sensitive fluorescent assay can be obtained on FluoroNunc white plates and modules. Furthermore, these products have the same quality of surface performance as all Immuno™ products. Existing chromogenic tests designed on PolySorp™ or MaxiSorp surfaces can therefore easily be transferred to fluorescence tests by using FluoroNunc plates or modules and simply changing the substrate to a fluorescent substrate offering an increased assay sensitivity.

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Fig. 1
Results of fluorescence assay

15 min; 1:500

ng/mL IgG	0.01	0.02	0.04	0.08	0.16	0.31	0.625	1.25	Bg
Mean	26.77	25.41	38.98	54.39	82.12	148.2	274.3	551.3	8.91
SD	11.11	5.253	13.45	14.24	7.941	6.554	16.32	28.32	4.77
2 SD	22.22	10.506	26.9	28.48	15.882	13.108	32.64	56.64	9.548
Mean + 2 SD	48.99	35.916	65.88	82.87	98.002	161.308	306.94	607.94	18.459
Mean - 2 SD	4.55	14.904	12.08	25.91	66.238	135.092	241.66	494.66	

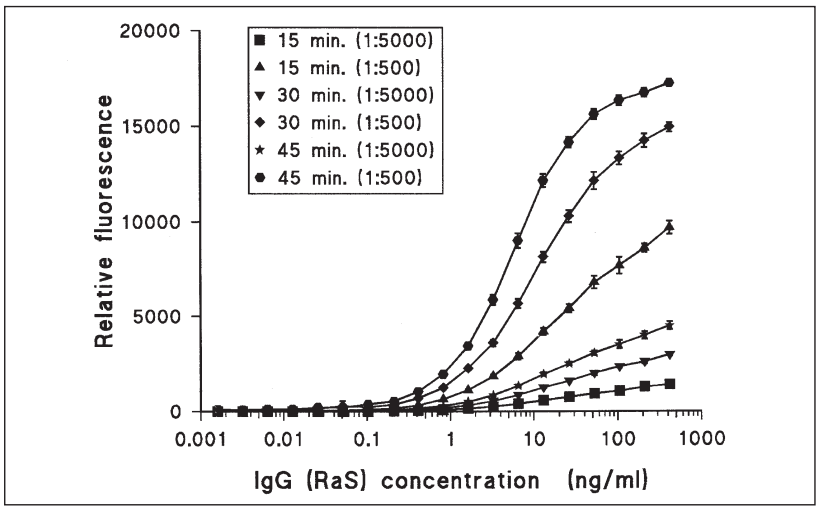
30 min; 1:500

ng/mL IgG	0.01	0.02	0.04	0.08	0.16	0.31	0.625	1.25	Bg
Mean	41.03	53.07	76.4	116.7	179.2	315.6	614.2	1145	21.21
SD	10.44	9.865	12.42	29.56	9.282	22.68	20.56	59.57	5.594
2 SD	20.88	19.73	24.84	59.12	18.564	45.36	41.12	119.14	11.188
Mean + 2 SD	61.91	72.8	101.24	175.82	197.764	360.96	655.32	1264.14	32.398
Mean - 2 SD	20.15	33.34	51.56	57.58	160.636	270.24	573.08	1025.86	

45 min; 1:500

ng/mL IgG	0.01	0.02	0.04	0.08	0.16	0.31	0.625	1.25	Bg
Mean	66.38	84.29	131	194.5	298.6	542	1018	1949	56.28
SD	18.35	21.22	38.62	50.23	17.88	40.8	39.1	75.32	16.54
2 SD	36.7	42.44	77.24	100.46	35.76	81.6	78.2	150.64	33.08
Mean + 2 SD	103.08	126.73	208.24	294.96	334.36	623.6	1096.2	2099.64	89.36
Mean - 2 SD	29.68	41.85	53.76	94.04	262.84	460.4	939.8	1798.36	

Fig. 2
Graphical representation



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