Fluorescent IEF-Marker

Application

IEF (Isoelectric Focusing) is a powerful analytical tool for the separation of ampholytes, mainly proteins. In order to ensure the high performance of analysis, standards of pI (pI markers) are needed. In addition to classical protein based standards, low molecular compounds were developed and successfully examined in capillary IEF and IEF-Gel electrophoresis. For capillary IEF UV absorption is the most popular method in use, on the hand UV induced fluorescence emission is of interest if derivatizations of proteins with e.g. dansyl chloride, fluorescamine, o-phtaldialdehyde or coumarin moieties are used to increase sensitivity.
An advantage for IEF gel electrophoresis with Fluorescent IEF-Marker is the possibility to control the formation of gradient without further staining (using illumination UV).

Fluorescent IEF markers can also be detected by UV-absorption at 280 nm (20°C), although the signal is not as strong as with fluorescence detection. The absorption maxima of the individual markers are between 308 and 350 nm. For fluorescence detection an excitation wavelength of 310 nm (individual excitation maxima: 310 to 400 nm) is suggested; the emission maximum of the individual markers lies between 410 and 500 nm.

Product Description

Stock Solution: 200 µl packages
Fluorescence: E_{max}, Exc. and conditions (see table 1)
concentration: 1, 2, 3 mg /ml
solution: aqueous (see table 1)
filtered through 0.45 µm membrane filter
Store at 4°C; stable for at least 6 months

Analysis Conditions for CIEF using Pressure Mobilization

Capillary:	neutral capillary
Anolyte:	91 mM phosphoric acid in gel buffer
Catholyte:	20 mM sodium hydroxide in water
Detection:	280 nm
Temperature:	20 °C
Injection:	20 psi 1 min
Polarity:	inlet anode, outlet cathode
Focussing voltage:	500 V/cm
Focussing time:	2 min
Mobilization:	0.5 psi, 500 V/cm anolyte -> catholyte Mobilization should be stopped after the last marker is eluted to avoid the filling of the capillary with anolyte.

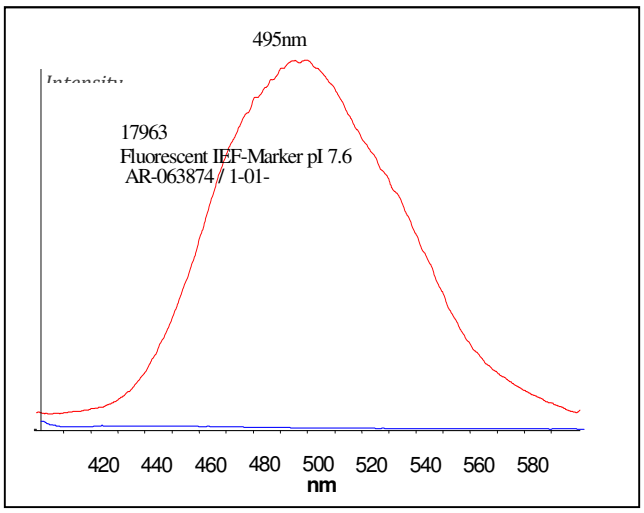

Figure 1: Emission spectrum and emission maxim of the Fluorescent IEF-Marker pI 7.6

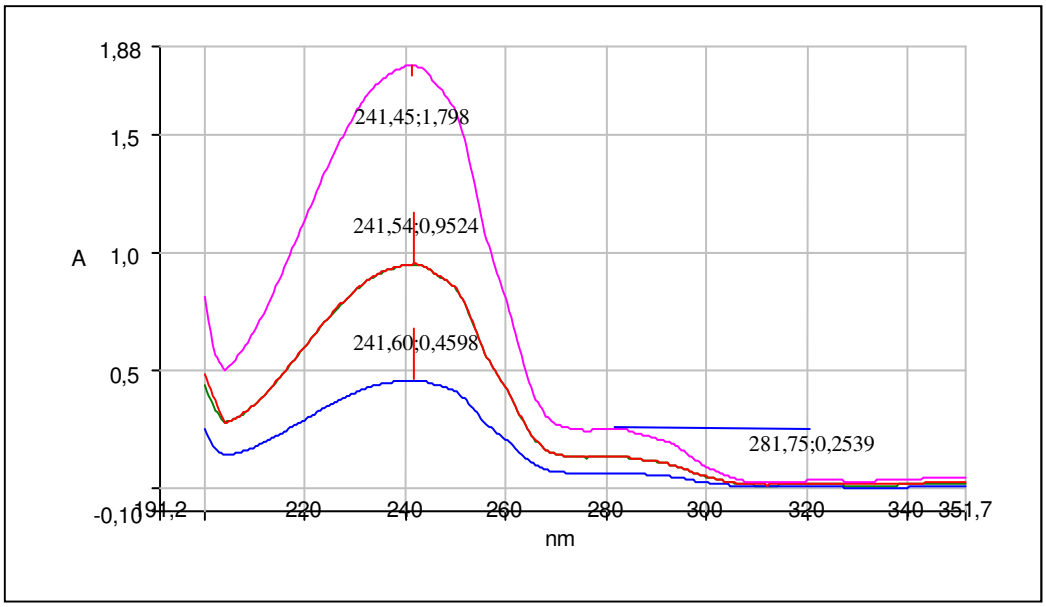

Figure 2: UV spectrum of the Fluorescent IEF-Marker pI 7.6 (three different concentration)

Product No.	Description	pI	Stock Solution Concentration in ultrapure water			Fluorescence		
			Marker [mg/ml]	HCl * [mM]	2-propanol [%]	Em_{max} [nm]	Exc. [nm]	Buffer for measurement pH =pI
74169	stock solution	2.1	3	-	50	430	340	50mM Citrate, 50mM KCl
72172	stock solution	3.0	2	5	50	445	360	0.1M Citrate
89827	stock solution	4.0	1	-	-	415	310	0.1M Citrate
89149	stock solution	5.2	1	5	-	424	336	0.1M Citrate
89478	stock solution	5.9	2	10	-	415	330	0.1M Citrate
77866	stock solution	5.5	3	15	-	412	325	0.1M Citrate
73938	stock solution	6.7	1	10	-	505	394	0.1M Phosphate
89508	stock solution	6.8	1	5	-	418	338	0.1M Phosphate
89951	stock solution	7.2	1	4	-	500	387	0.1M Phosphate
89952	stock solution	7.6	1	10	-	495	385	0.1M Tris
75734	stock solution	8.1	3	-	-	420	340	0.1M Tris
89357	stock solution	8.7	1	10	-	500	390	0.1M Tris
90699	stock solution	9.0	1	10	-	495	385	0.1M Tris
89268	stock solution	9.5	3	8	-	415	325	0.1M Carbonate
77672	stock solution	10.3	1	10	-	495	388	0.1M Carbonate
17951	stock solution of Marker-Mix		2 (of each)	5	50	-	-	-

Table 1: conditions of the stock solution from Fluorescent IEF-Markers

Directions for the use of Fluorescent IEF-Markers

Protocol for the use of Fluorescent IEF-Marker
Prepare the IEF-marker stock solution by dissolving the substance in the solvent according to the table 1. Sonicate for up to 10 min if necessary. Store at 4 °C (the solution is stable for approximately 6 months). For use in HPCE this stock solution should be diluted 1:100 in a suitable ampholyte solution (2% ampholyte 3-10; e.g. 10046). For uses in IEF-gels the stock solution can be loaded directly onto the gel (max. 1 µl per lane).

Reference:

M. Horka, Th. Willimann, M. Blum, P. Nording, Z.Friedl, K. Slais, Capillary isoelectric focusing with UV-induced fluorescence detection, J. of Chromatography A, 916 65-71 (2001)

Precautions and Disclaimer:
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.