Recent development of Fluorescence Polarization Immunoassays for food contaminants

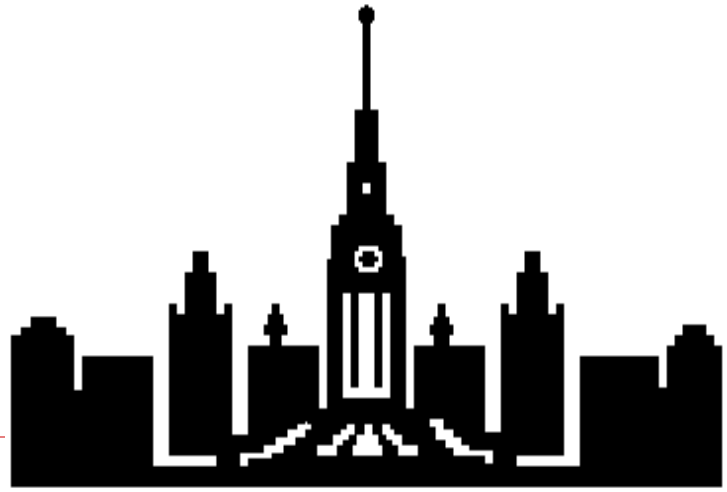
4th International Conference and Exhibition on Food Processing & Technology
"AgriFood Antibodies - Immunoassays for Food Safety and Quality Control"
10 August 2015, London, UK

Prof. Sergei A. EREMIN

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A.N.Bakh Institute of Biochemistry of Russian Academy of Science, Moscow, Russia

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http://www.enzyme.chem.msu.ru/eremin/



V.Lomonosov
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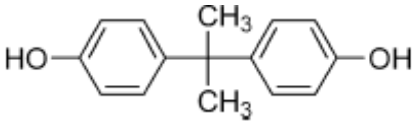


Introduction for contamination

The contamination environmental and food samples with contaminants maybe vary from low or "zero" levels up to very high concentration up to µg/ml.

The number of contaminants and their metabolites presented in samples may range from one to dozen and their distribution in different types of sample may also be very variable.

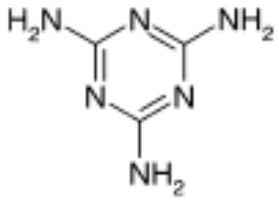
Food contaminants



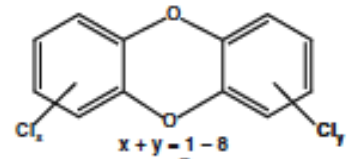
Bisphenol A

Chemical

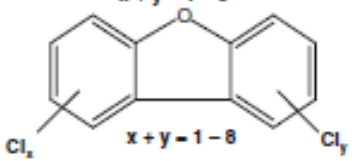
- Pesticides
- Drugs
- Detergents
- toxins



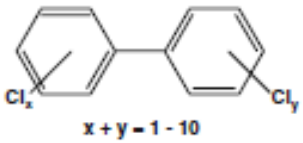
Melamine



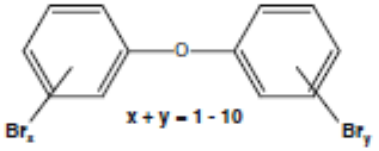
Polychlorinated dibenzo-*p*-dioxins (PCDDs)



Polychlorinated dibenzofurans (PCDFs)



Polychlorinated biphenyls (PCBs)



Polybrominated diphenyl ethers (PBDEs)

Biological

- Bacteria
- Microorganisms

Requirements for detection of chemical contaminants

- Simple
- Quick
- Precise
- Sensitive
- Multi detection

Tendency in Analytical Detection from Chromatography to Immunoassay

- HPLC
- GC
- GC-MS



- Immunoassay

- Labor intensive
- Complicated cleanup
- Expensive equipment
- Time consuming

High sensitivity
High specificity
Rapid and simple
High Throughput Screening

Immunoassay

Immunochemical methods are based on the reversible binding of an antigen to specific antibodies, which are specially prepared for a given analyte:



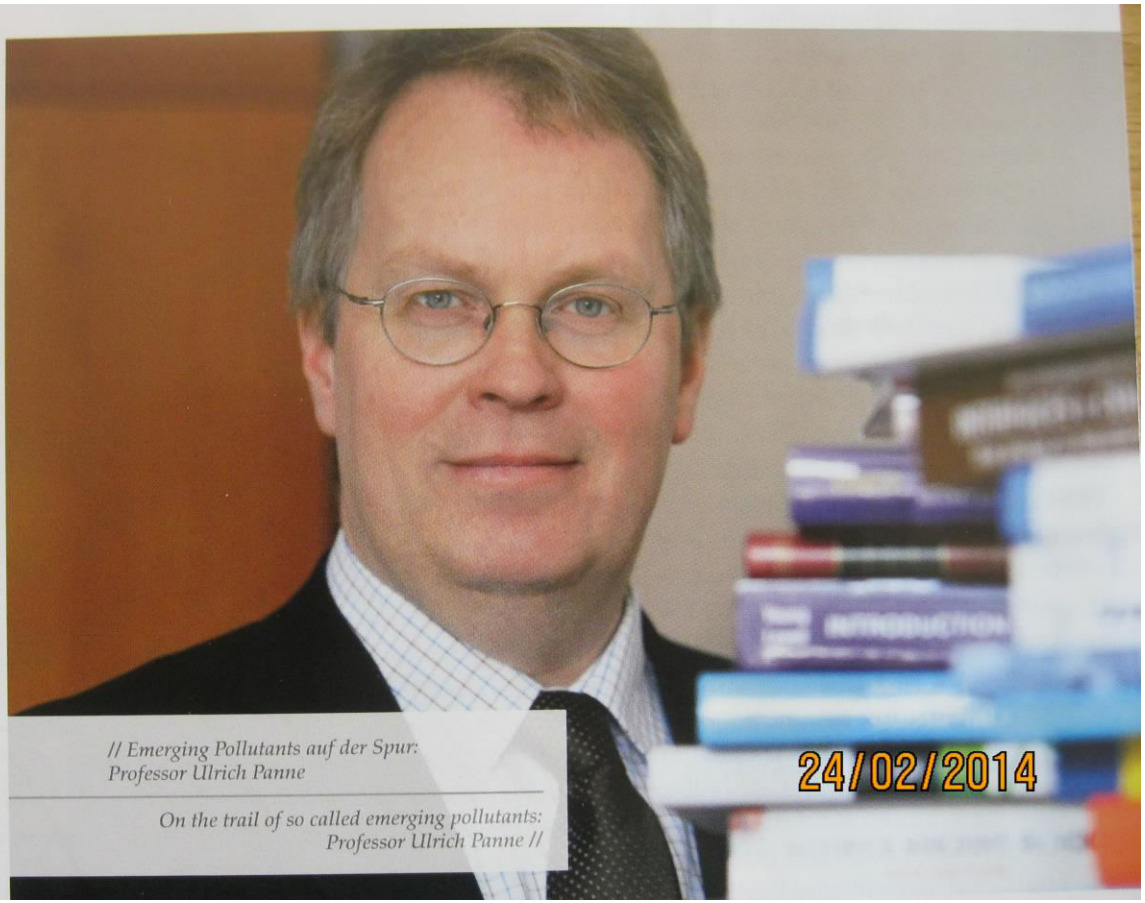
where Ab is a specific antibody, Ag is an antigen, Ag : Ab is an antigen–antibody immune complex, and *K*as is the constant of formation of the complex.

"The advantage of Immunoassays is that they provide fast and low cost analyses of many samples" – Ulrich Panne

Director, Federal Institute for Materials Research and Testing (BAM), Berlin, Germany

boldt-Universität zu Berlin and Director of BAM Department 1. One of his key research fields deals with immunoassays, or methods serving to determine even the tiniest quantities of bioactive substances. "The advantage of immunoassays," explained Panne, "is that they provide fast and low cost analyses of many samples."

The methods developed by BAM can be applied in many ways. Whether it's about detecting aflatoxin on ice cream or mycotoxins in flour, or whether manufacturers of generics want to ensure that every pill contains exactly the same agent – analytics provides the key each



Immunoassay

enzyme-linked immunosorbent assay (ELISA)

lateral flow immunoassay (strip-test)

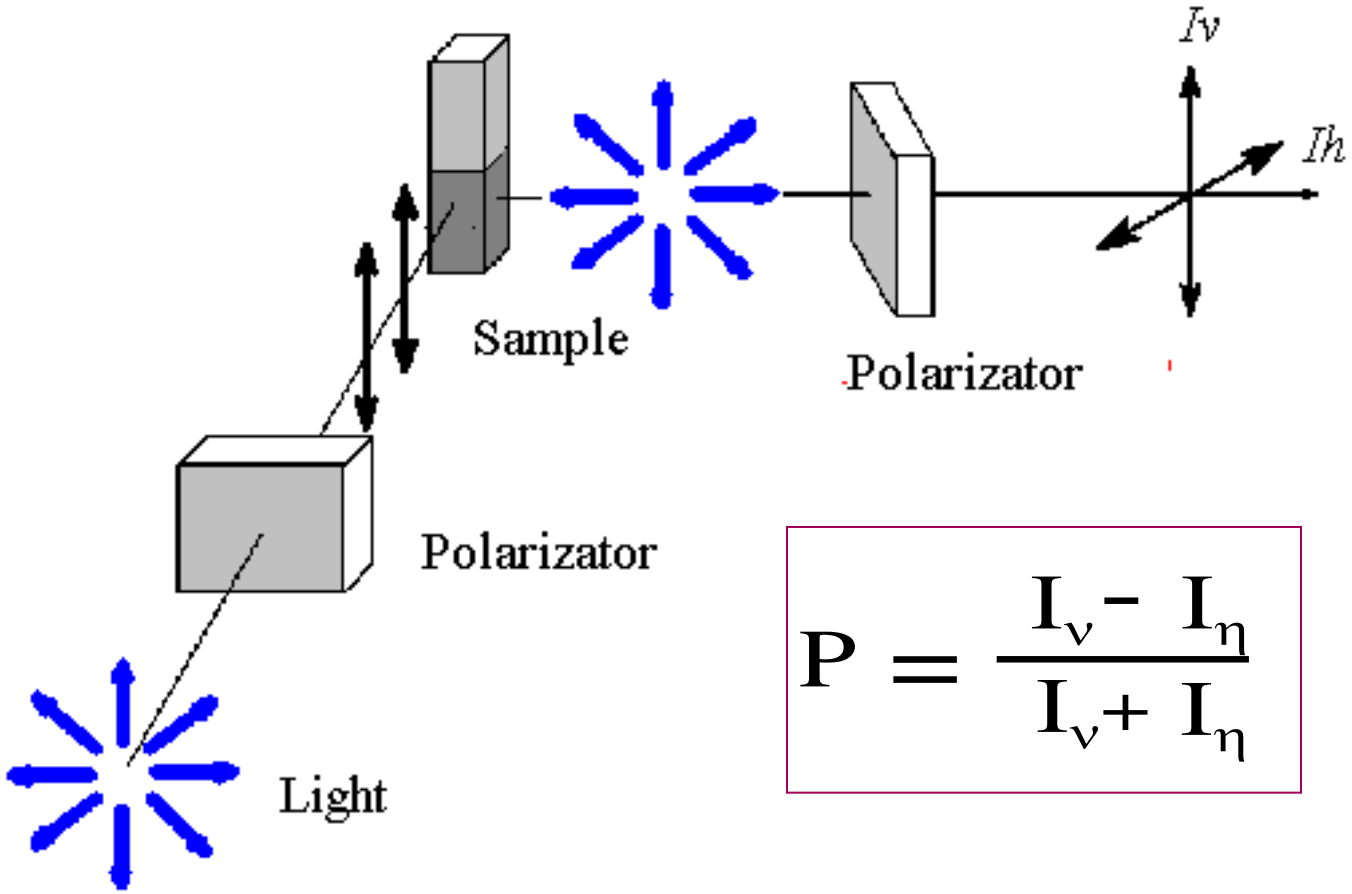
fluorescence polarization immunoassay (FPIA)

FPIA

Fluorescence Polarization Immuno Assay

In the immunoassay method, fluorescence polarization immunoassay (FPIA) is the most extensively used homogeneous technique, which meets the requirements of a simple, reliable, fast and cost-effective analysis

Detection of Fluorescence Polarization



$$P = \frac{I_\nu - I_\eta}{I_\nu + I_\eta}$$

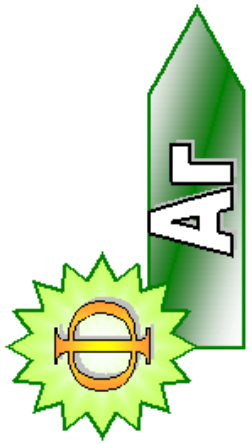
F. Perrin, *J. Phys. Radium*, 7 (1926) 390-401.

[1] Perrin (8) showed in his equation that the P value can be expressed as a function of such factors as temperature and viscosity together with molecular parameters as follows.

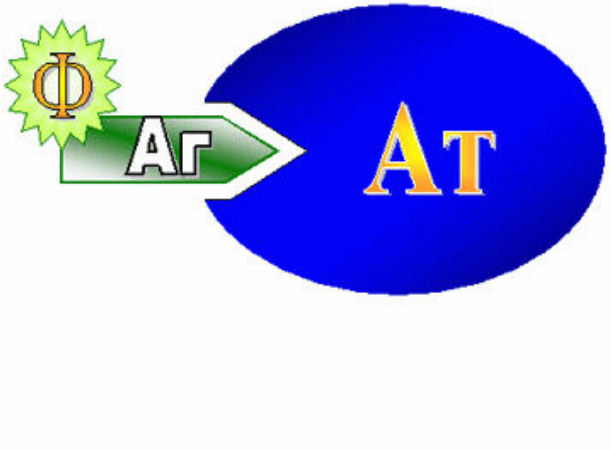
$$\frac{1}{P} = \frac{1}{P_0} + \left(\frac{1}{P_0} - \frac{1}{3}\right) \times \left(\frac{RT}{V}\right) \frac{\tau}{\eta} \qquad (2)$$

P	observed value of fluorescence polarization
Po	a constant (maximal value of P obtained in a rigid medium)
R	gas constant
T	absolute temperature
η	viscosity (poise)
r	relaxation time of fluorescence excitation(s)
V	molecular volume

Principle of Fluorescence Polarization



$$P = \frac{I_v - I_h}{I_v + I_h}$$



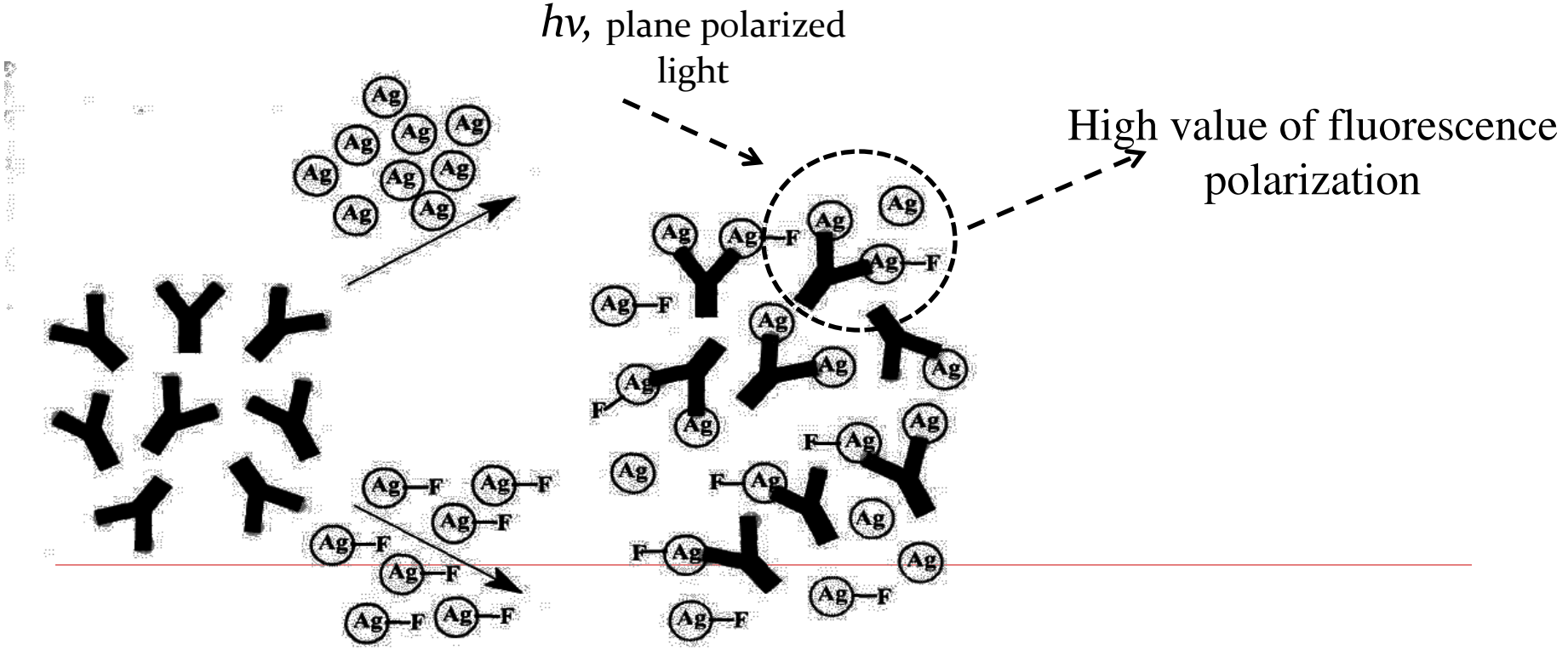
Fast rotation
Low Fluorescence Polarization

Slow rotation
High Fluorescence Polarization

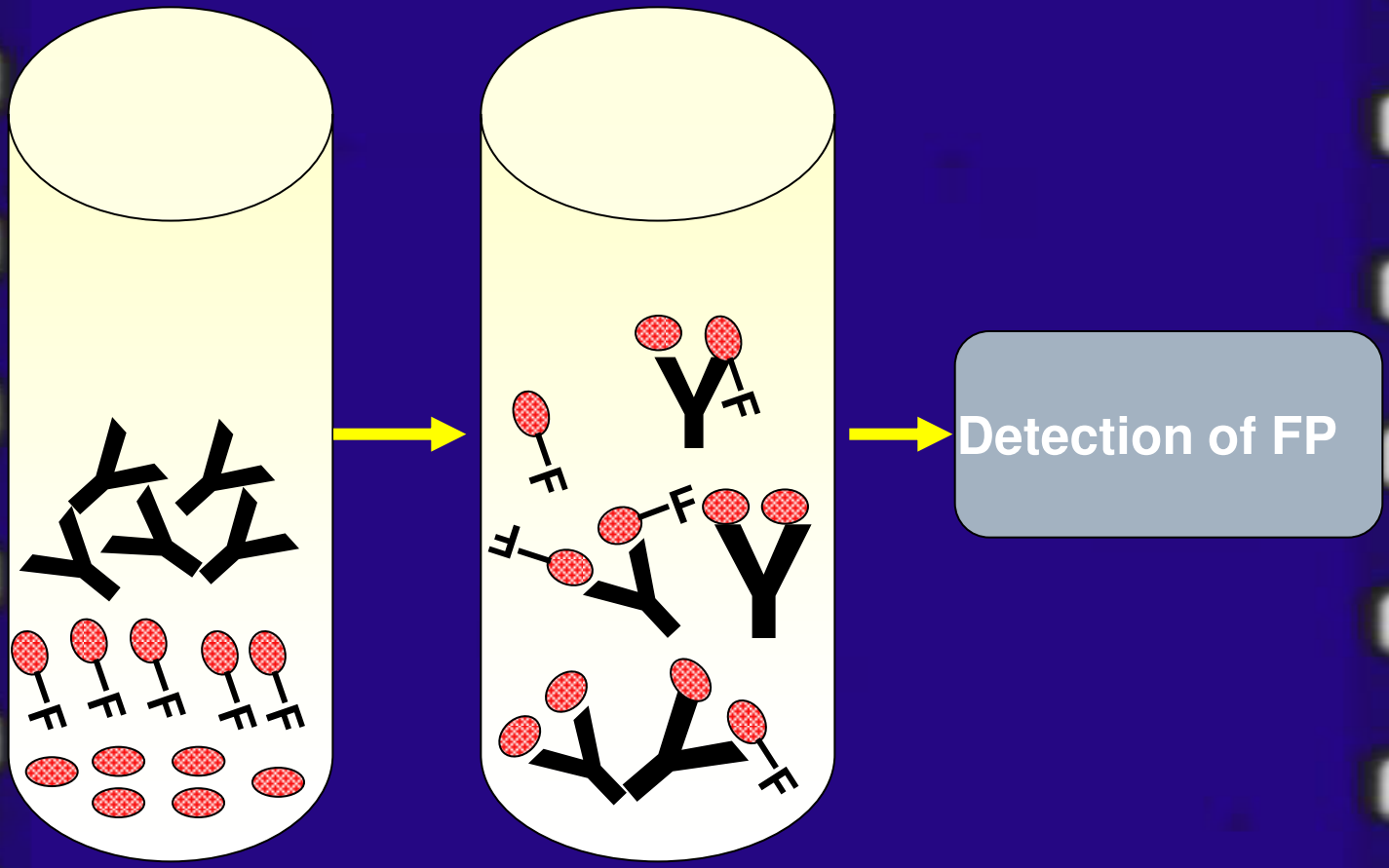
Principle of FPIA competitive immunoassay with separation of free and bond tracer

Ag – antigen, *Ab* - antibody

$$Ag + Ab \rightleftharpoons AgAb$$



Performance of FPIA



David S. Smith and Sergei A. Eremin. Fluorescence polarization immunoassays and related methods for simple, high-throughput screening of small molecules. Anal. Bioanal. Chem., 391(5), 1499-1507 (2008).

Anal Bioanal Chem (2008) 391:1499–1507
DOI 10.1007/s00216-008-1897-z

REVIEW

Fluorescence polarization immunoassays and related methods for simple, high-throughput screening of small molecules

David S. Smith · Sergei A. Eremin

Received: 5 December 2007 / Revised: 14 January 2008 / Accepted: 17 January 2008 / Published online: 10 February 2008
© Springer-Verlag 2008

Sergei Eremin more cited paper

Fluorescence Polarization ImmunoAssay (FPIA)

ADVANTAGES

No separation steps
No washing steps
Simple and quick
Cost effective
Stable tracer
High precision
Stability of the standard curve

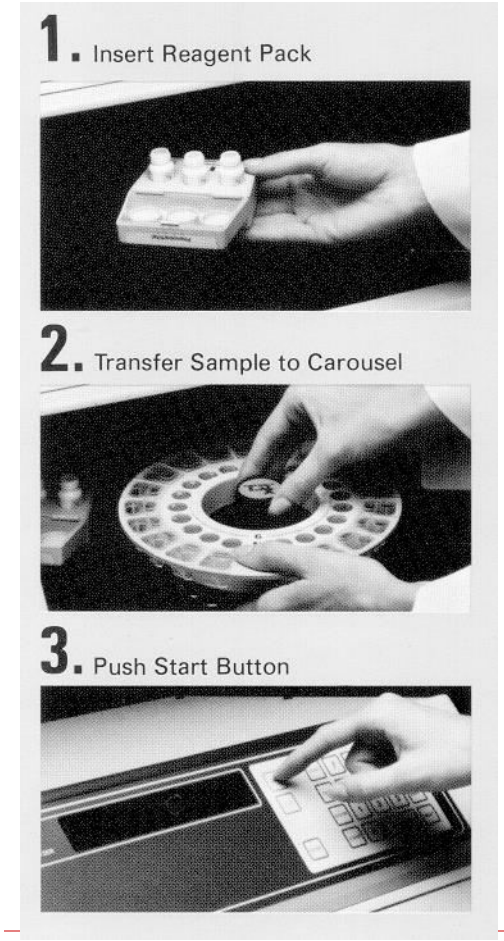
LIMITATIONS

Only for small molecules
Lower sensitivity than ELISA
Matrix dependent
Special instrument

TDxFLx Analyzer (Abbott Lab., USA)



Automated method for FPIA using TDxFLx Abbott (USA)



1. Insert reagent pack

2. Load carousel with samples (100-500 ul)

3. Push button and receive results after 5-20 min

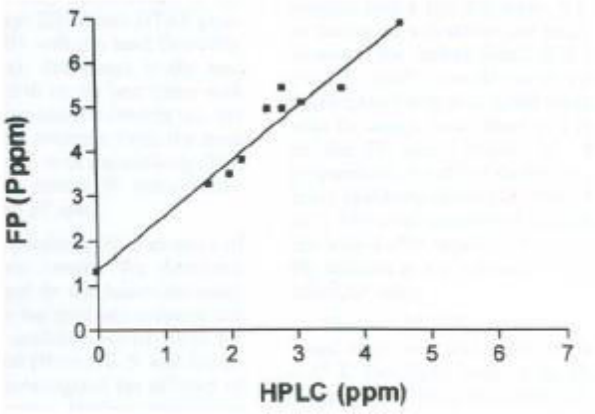
Portable FP instrument – Sentry-200
Distributor in China - Wang Dong

ebiotek@gmail.com 133-8113-8788

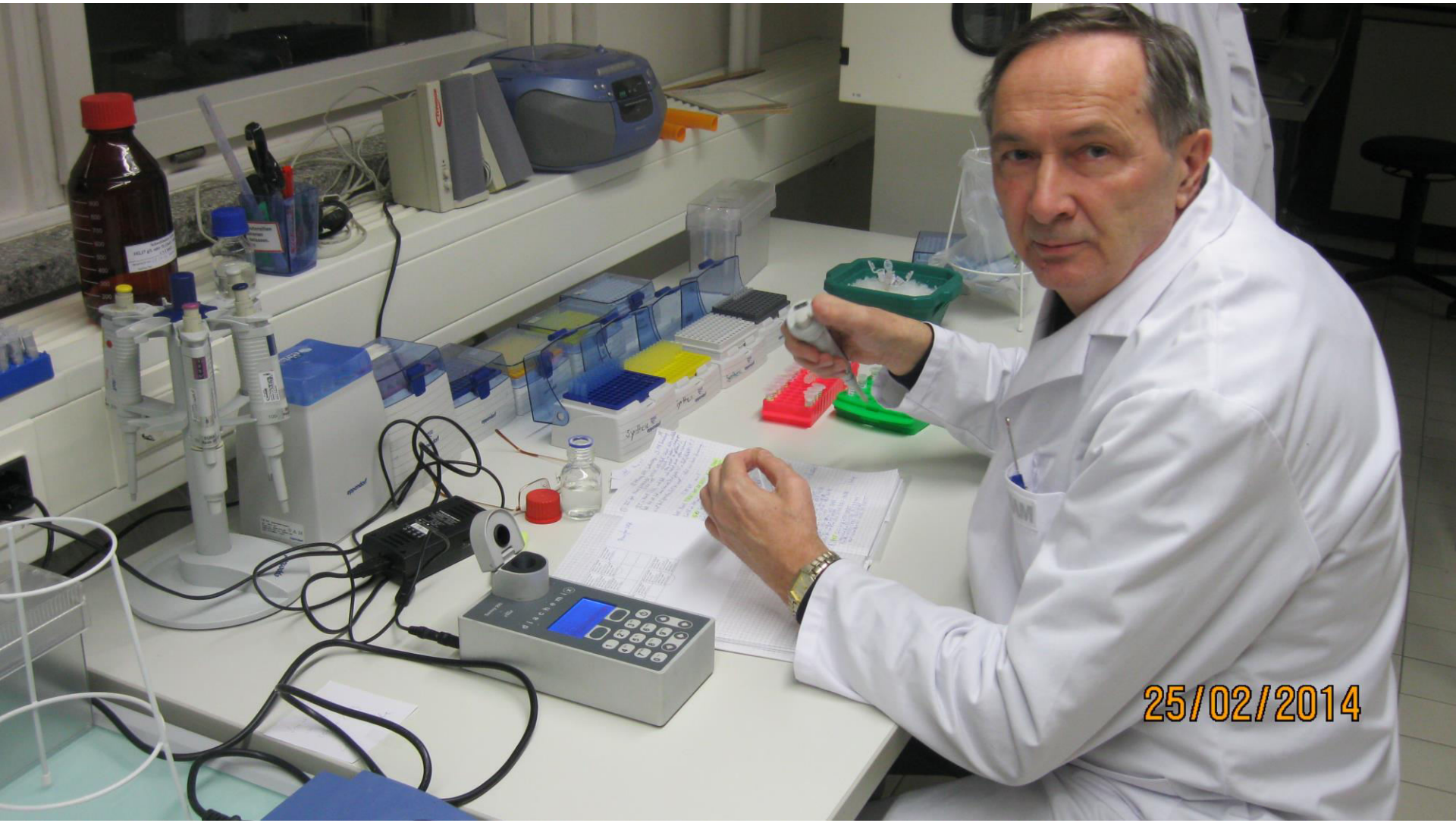


Sentry FP
(Diachemix Corp,
& Cape Cod Assoc.)

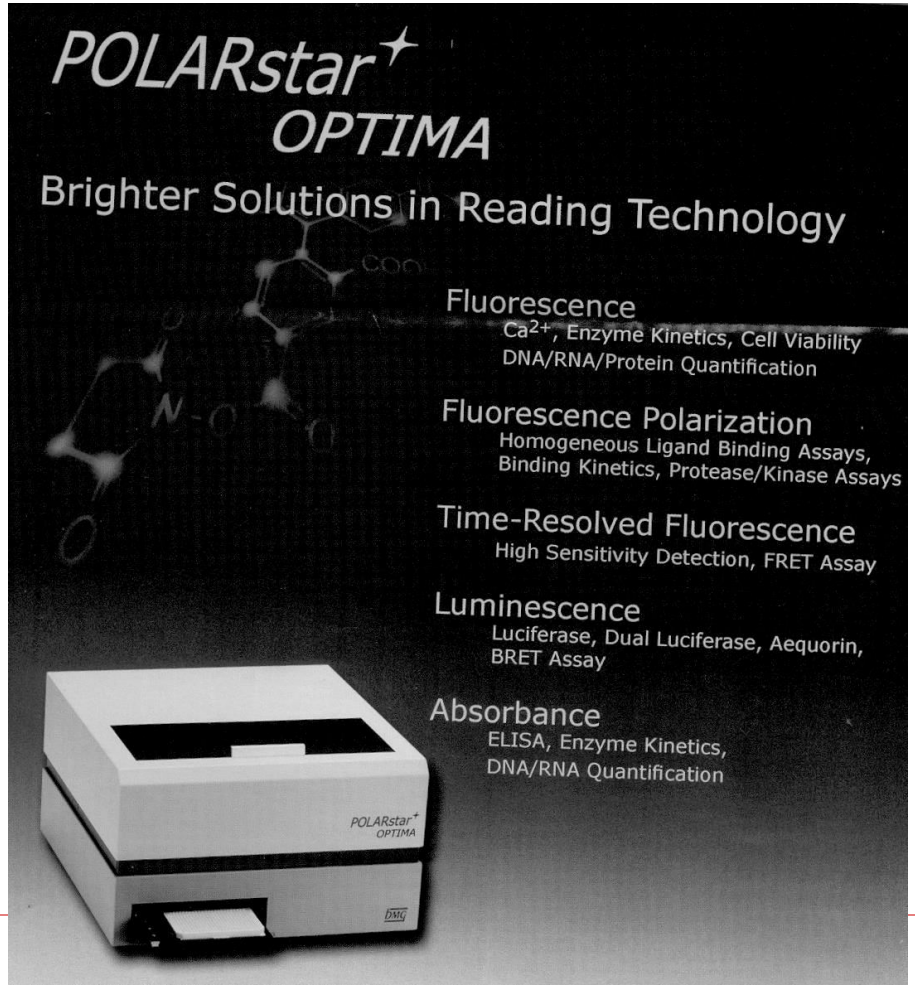
Assays for:
Deoxynivalenol
Fumonisins
Aflatoxin B_1
Zearalenone



BAM, Berlin, Germany



POLARstar (DMG, Germany)

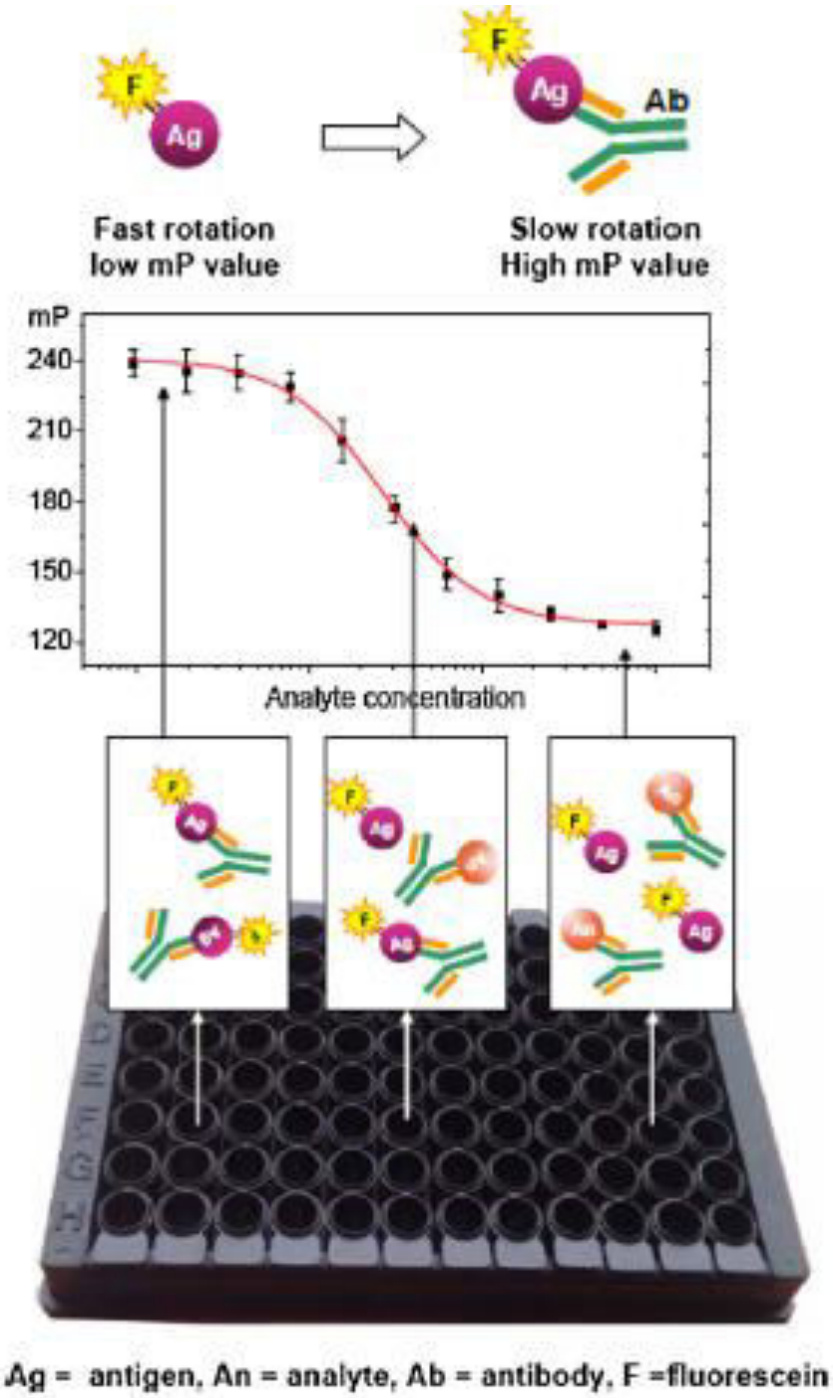


Fluorescence Polarization Immuno assay (FPIA)

Method FPIA

Load:
10 uL sample
0.1 mL tracer solution
0.1 mL antibody
mix and measure mP

Total time for assay 1 plate
Low than 10 min



Ag = antigen, An = analyte, Ab = antibody, F =fluorescein

FP instrumnet

ellie

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U.S.A.
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www.ellielab.com
(800) 556-6953

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Payment Terms

Item	Description	Qty	Rate	Total
S200	Sentry 200 Portable FP Reader	3	7,500.00	22,500.00
B1001-1000 A	Brucella FPA 1000 Tests	5	1,300.00	6,500.00
D1001	Deoxynivalenol qFPA Test Kit 100 tests	5	680.00	3,400.00
E1001-1000	Equine Infectious Anemia FPA Test Kit 1000 Tests	5	1,300.00	6,500.00
S200S	Sentry 200S 12-Well FP Intrument		10,500.00	10,500.00
		Total		$49,400.00

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PRODUCT INFORMATION SHEET

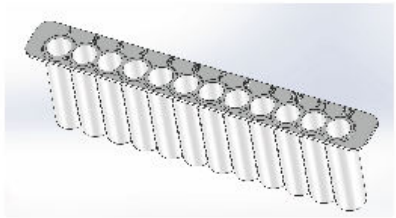
Sentry 2000S

Fluorescence polarization reader for 12-well strips

- Fast accurate reader for 12 samples
- Read time less then one second per well
- Equipped with an injector for automated operation
- Injector precision highest in the industry with CV of 1% when delivering 10 microliter tracer.
- Easy software operation directly from Microsoft® Excel®
- Adjustable read precision
- USB connectivity
- Lower than 1 mP standard deviation between readings
- Sensitivity 1 pM
- Built in LCD Polarizer switch for fast polarization switching and fast operation



Stripwells



Sentry 2000S works with black strips with 12 wells which are custom made and hold 1 ml of liquid or with standard 12-well or 8-well microplate strips.

Ordering Information

Catalog #	Description
S2000S	12-well FP Reader
S2000Si	12-well FP Reader with one injector
STRIPS	1 ml black 12-well strips
STRIPS8	8-well microplate strips
STRIPS12	12-well microplate strips
STBG	Tubing for reagent dispensing with needle
SINTBG	Internal tubing set for S2000S
SINSERT8	Aluminum adapter for 8-well microplate strips
SINSERT12	Aluminum adaper for 12-well microplate strips
SCOMP	Sentry Laptop for instrument control

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FPIA development and optimization

- **Our work on FPIA was focused on improvement of FPIA characteristics mainly sensitivity**

- **FPIA consist of:**

  Antibody (Ab), Antigen (Ag) and the Tracer (Ag-F)

- **It is based on the competitive binding of antigen to the antibody:**

$$Ab + Ag\text{-}F \underset{}{\overset{Kd_1}{\rightleftharpoons}} Ab{:}Ag\text{-}F \qquad Kd_1 = \frac{[Ab]\cdot[{}^*A]}{[Ab - {}^*A]}$$

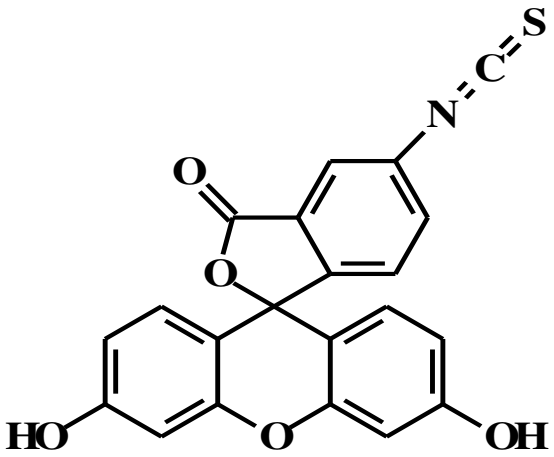
Kaff=1/Kd

$$Ab + Ag \overset{Kd_2}{\rightleftharpoons} Ab{:}Ag \qquad Kd_2 = \frac{[Ab]\cdot[A]}{[Ab - A]}$$

- **The improvement in sensitivity demands:**

- **The use of low antigen concentration, requires high affinity constant antibodies (Kaff)**
- **The antigen must compete binding of tracer. The two constants Kd_1 and Kd_2 should be similar**

Fluorescein derivatives with active group and Synthesis of tracers (fluorescein labeled antigen)

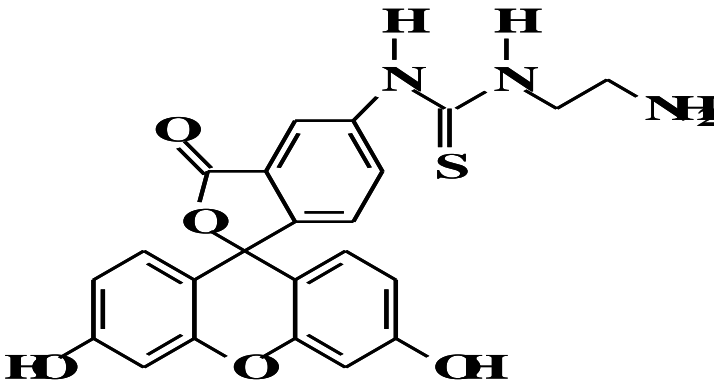


FITC

Ag-NH_2 + FITC → Ag-NH-CS-NH-Fluorescein

NH_2-CH_2CH_2-NH_2 + FITC →

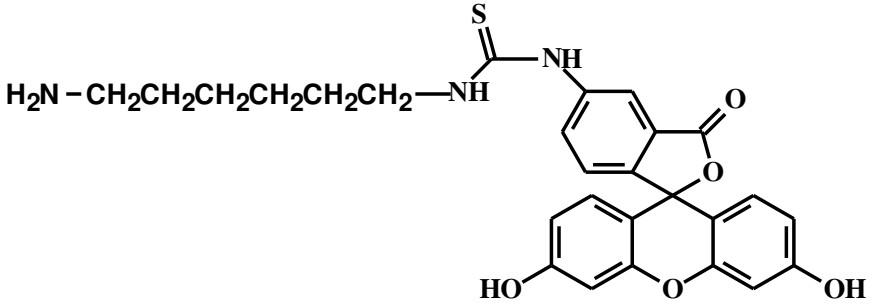
NH_2-CH_2CH_2-NH-CS-NH-Fluorescein (NH_2-EDF)



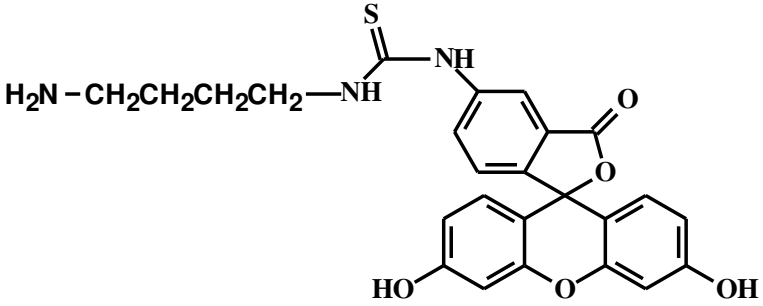
EDF

Ag-COOH => Ag-CO-Z

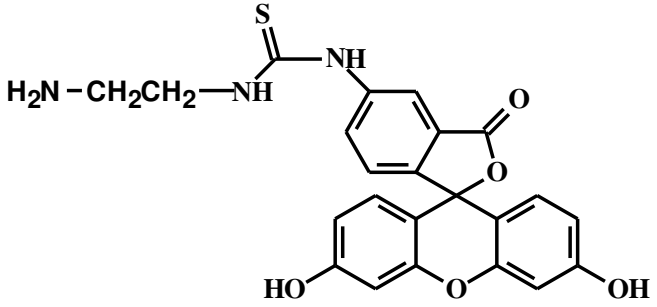
Ag-CO-Z + NH_2-EDF = Ag-CO-NH-EDF



HDF

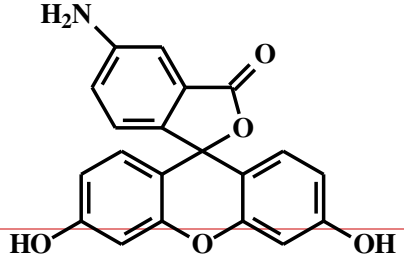


BDF

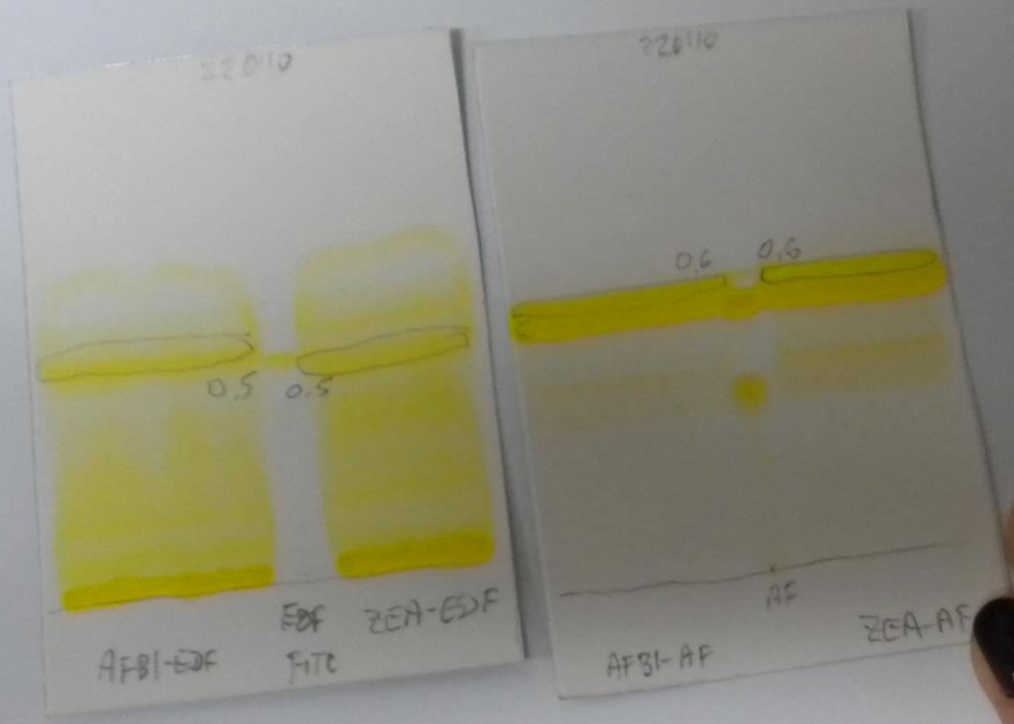


EDF

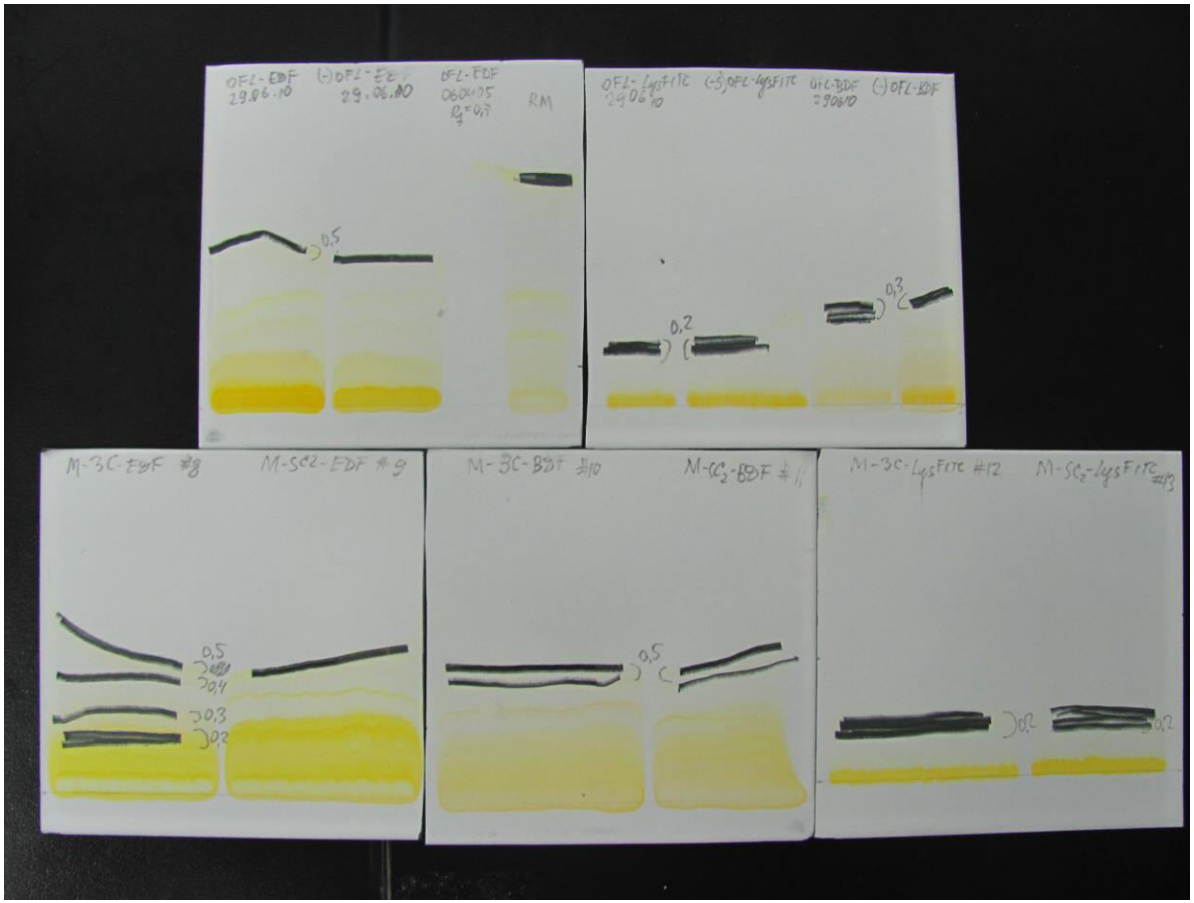
Amino-Fluorescein derivatives
NH2-(CH2)n-Fluorescein
n = 6, 4, 2, 0



AF


0,5
0,5
AFB1-EDF
EDF
FITC
ZEA-EDF
0,6
0,6
AF
AFB1-AF
ZEA-AF

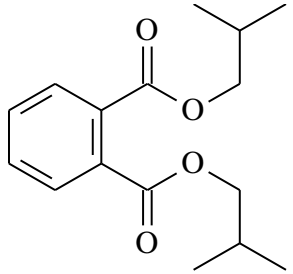
TLC purification for tracer

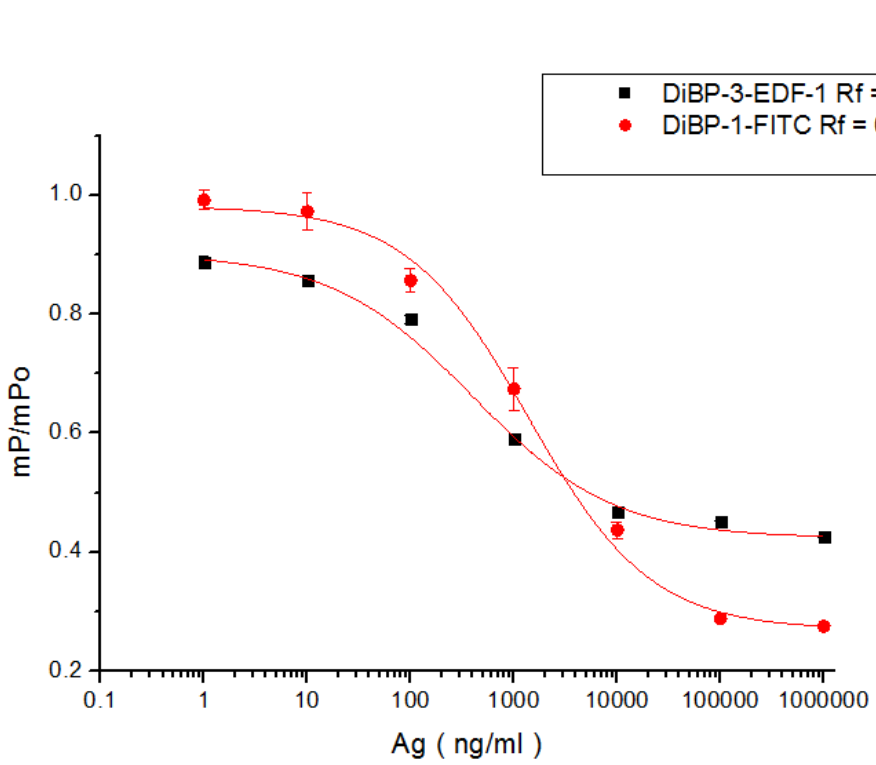


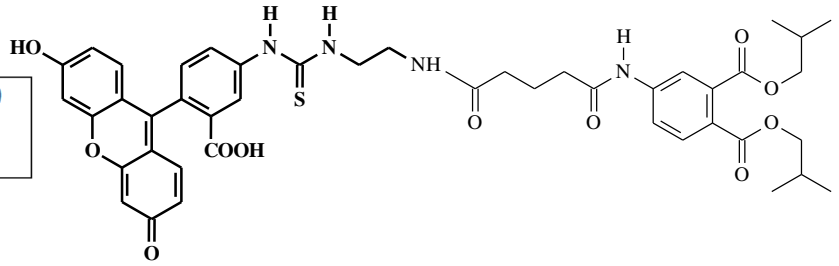


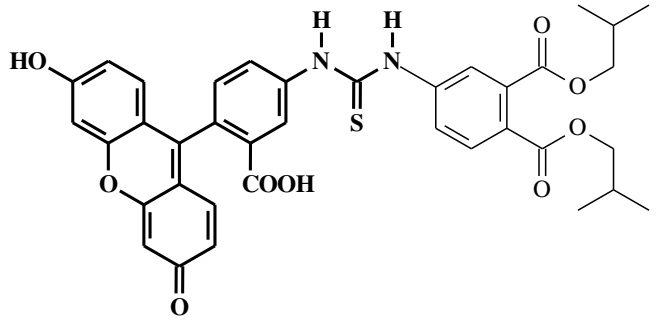
FPIA for DiBP (Di-iso-Buthyl-Phthalate)

GDUT, 15 July 2015

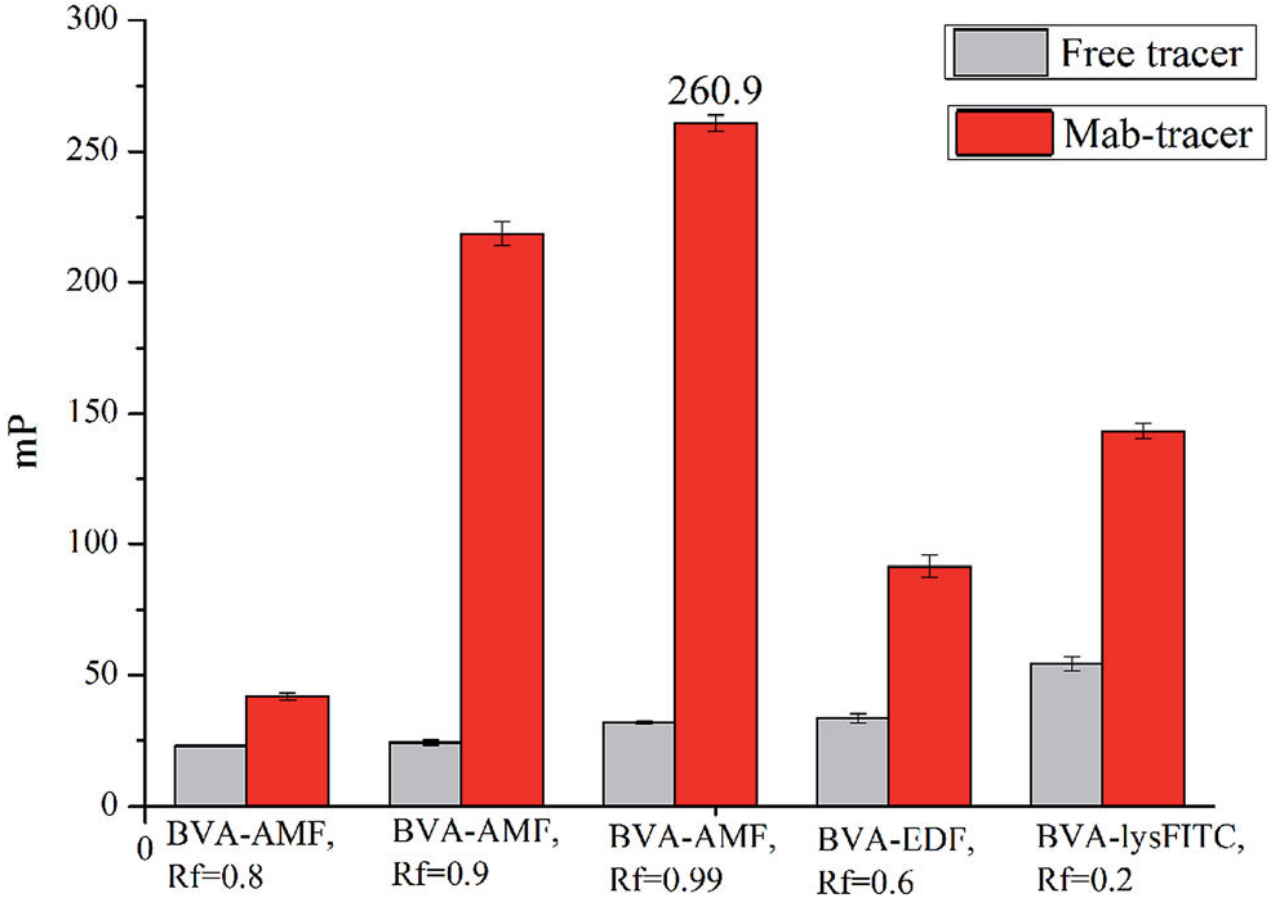






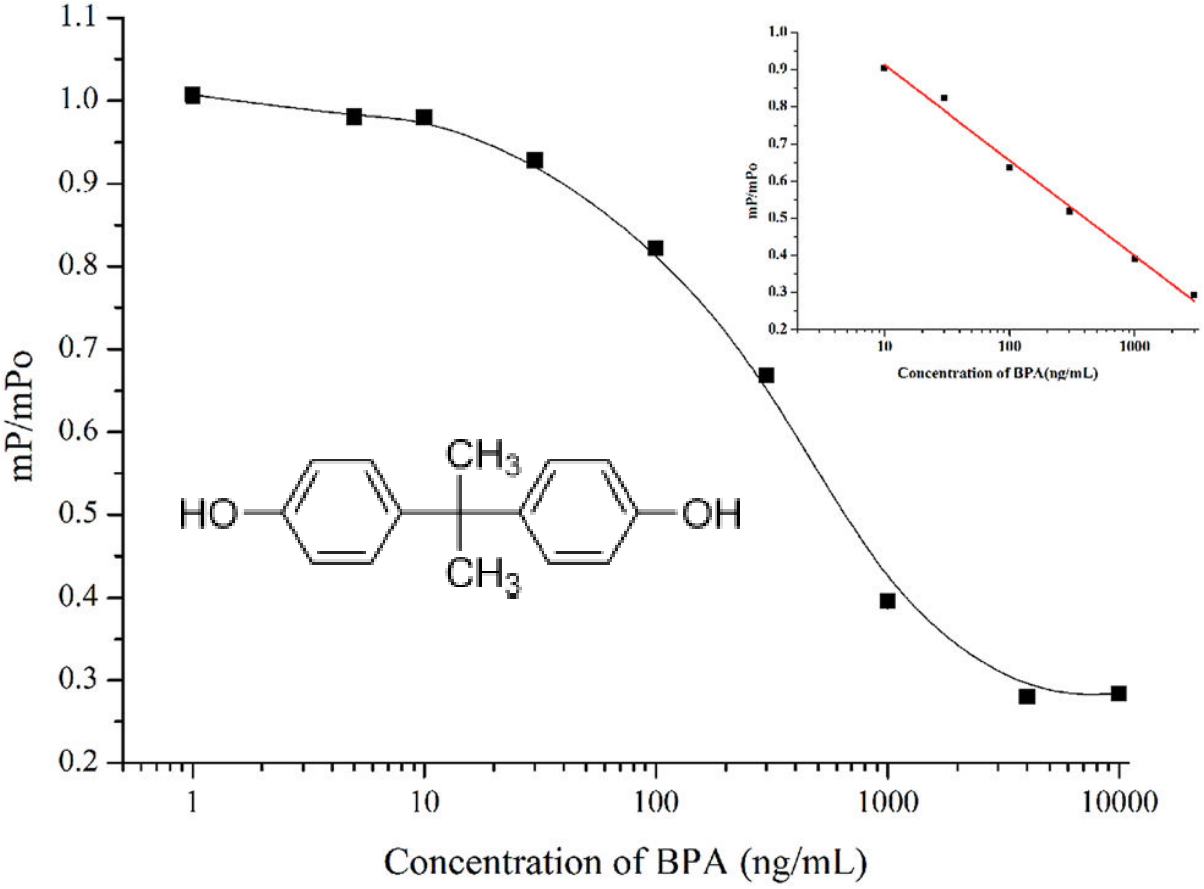


Pinong Huang, Suqing Zhao, Sergei A. Eremin, Shengwu Zheng, Dan Lai, Yingshan Chen and Bin Guo. A fluorescence polarization immunoassay method for detection of the bisphenol A residue in environmental water samples based on a monoclonal antibody and 4′-(aminomethyl)fluorescein. Anal. Methods, 7(10), 4246-4251 (2015). DOI: 10.1039/C5AY00818B



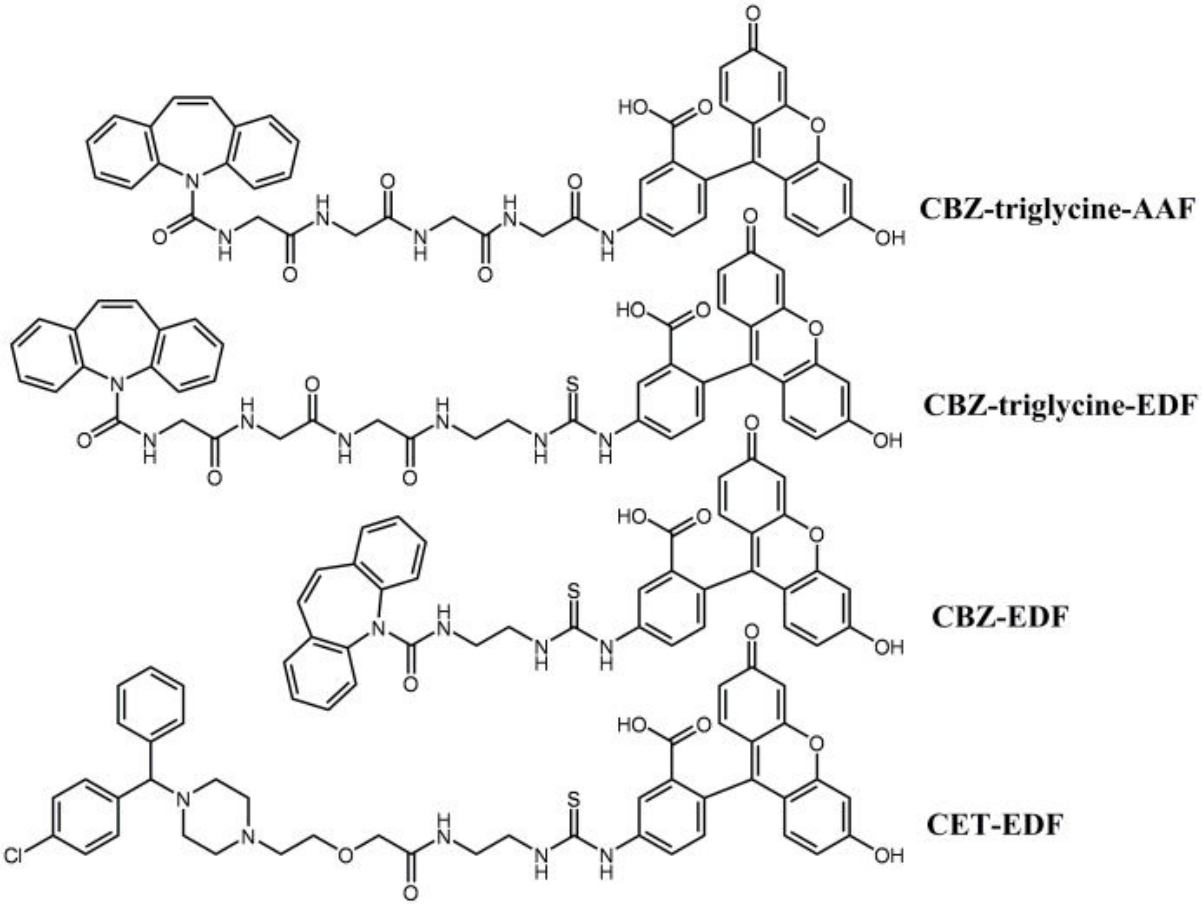
The binding of 5B8 MAb with the BVA–AMF tracer in BB working solution

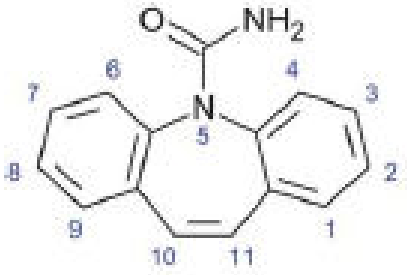
Pinong Huang, Suqing Zhao, Sergei A. Eremin, Shengwu Zheng, Dan Lai, Yingshan Chen and Bin Guo. A fluorescence polarization immunoassay method for detection of the bisphenol A residue in environmental water samples based on a monoclonal antibody and 4'-(aminomethyl)fluorescein. Anal. Methods, 7(10), 4246-4251 (2015). DOI: 10.1039/C5AY00818B



FPIA calibration curve and linear fitting for BPA using the 5B8 MAb and BVA–AMF tracer

Lidia Oberleitner, Sergei A. Eremin, Andreas Lehmann, Leif-Alexander Garbe,Rudolf J. Schneider. Fluorescence Polarization Immunoassays for Carbamazepine – Comparison of Tracers and Formats. Anal. Methods, (2015). DOI: 10.1039/C5AY00818B



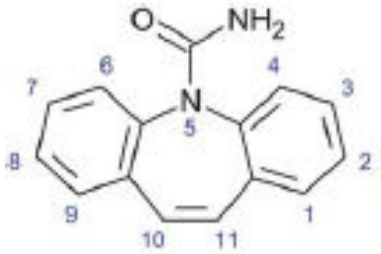


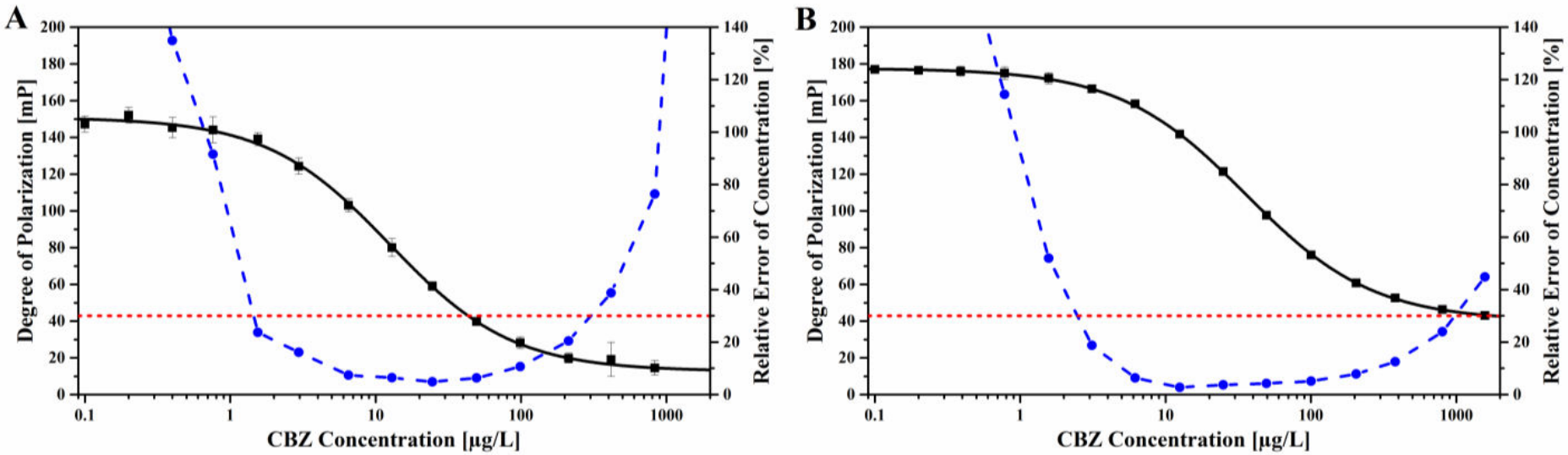
Carbamazepine

The measurement of an individual sample can be carried out within 4 min

Lidia Oberleitner, Sergei A. Eremin, Andreas Lehmann, Leif-Alexander Garbe,Rudolf J. Schneider. Fluorescence Polarization Immunoassays for Carbamazepine – Comparison of Tracers and Formats. Anal. Methods, (2015). DOI: 10.1039/C5AY00818B

Carbamazepine
main drug contaminants in river water





CBZ FPIA calibration curves (black solid lines), precision profiles (blue dashed lines) and measurement ranges (intersec-tion points at 30 % relative error of concentration, dotted red lines) determined on MTP (A) and in tubes (B).

Jin-Yi Yang, Yan Zhang, Hong Wang, Zhen-Lin Xu, Sergei A. Eremin, Yu-Dong Shen, Qing Wu, Hong-Tao Lei & Yuan-Ming Sun. Development of fluorescence polarisation immunoassay for carbofuran in food and environmental water samples. Food Agric. Immunol. 26(3), 340-355 (2015).

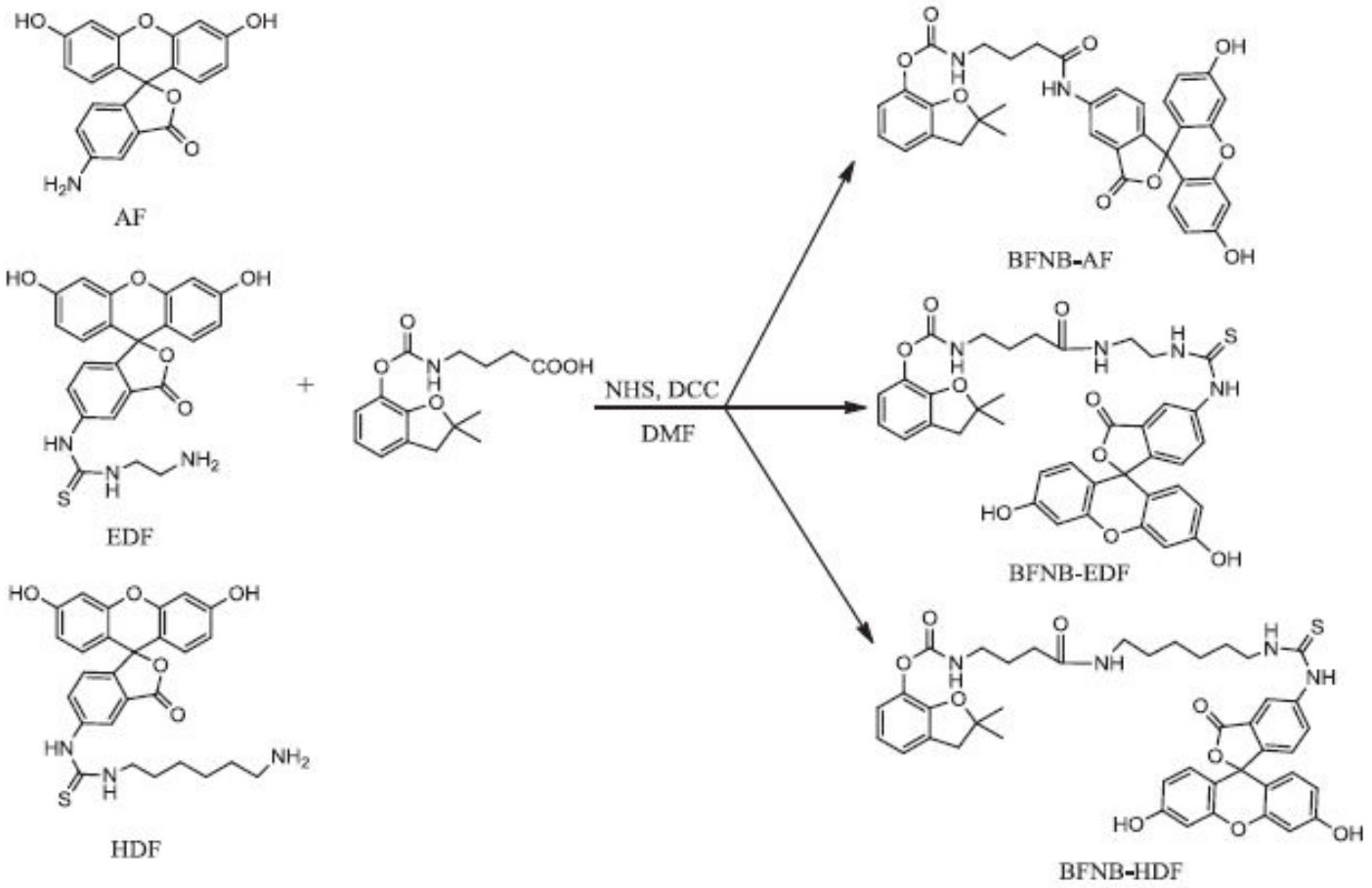


Figure 1. Synthesis process of three tracers (BFNB-AF, BFNB-EDF and BFNB-HDF).

Jin-Yi Yang, Yan Zhang, Hong Wang, Zhen-Lin Xu, Sergei A. Eremin, Yu-Dong Shen, Qing Wu, Hong-Tao Lei & Yuan-Ming Sun. Development of fluorescence polarisation immunoassay for carbofuran in food and environmental water samples. Food Agric. Immunol. 26(3), 340-355 (2015).

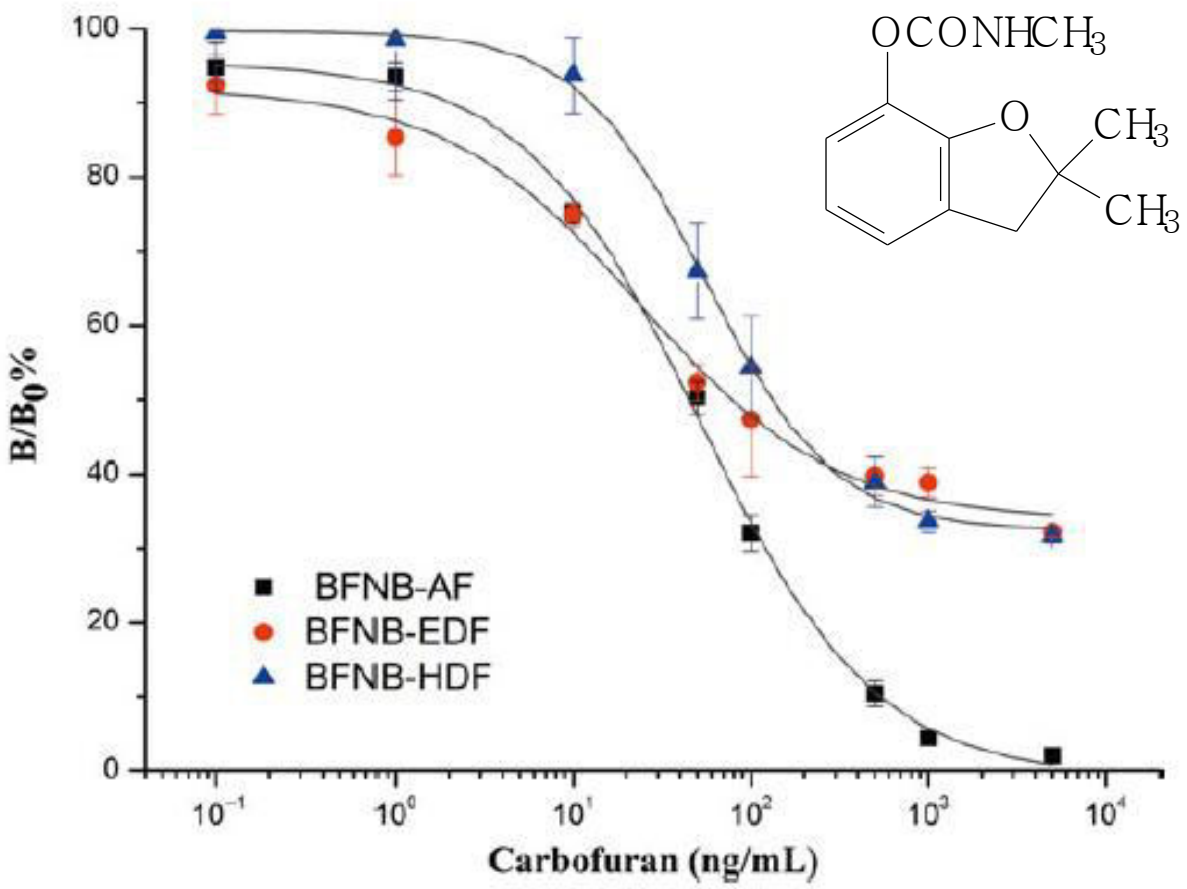
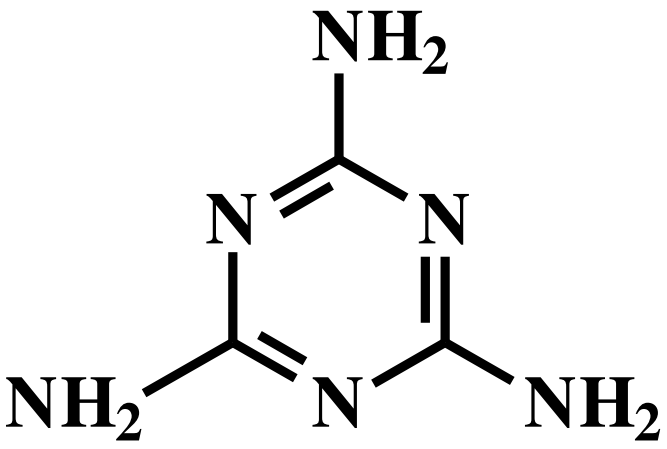
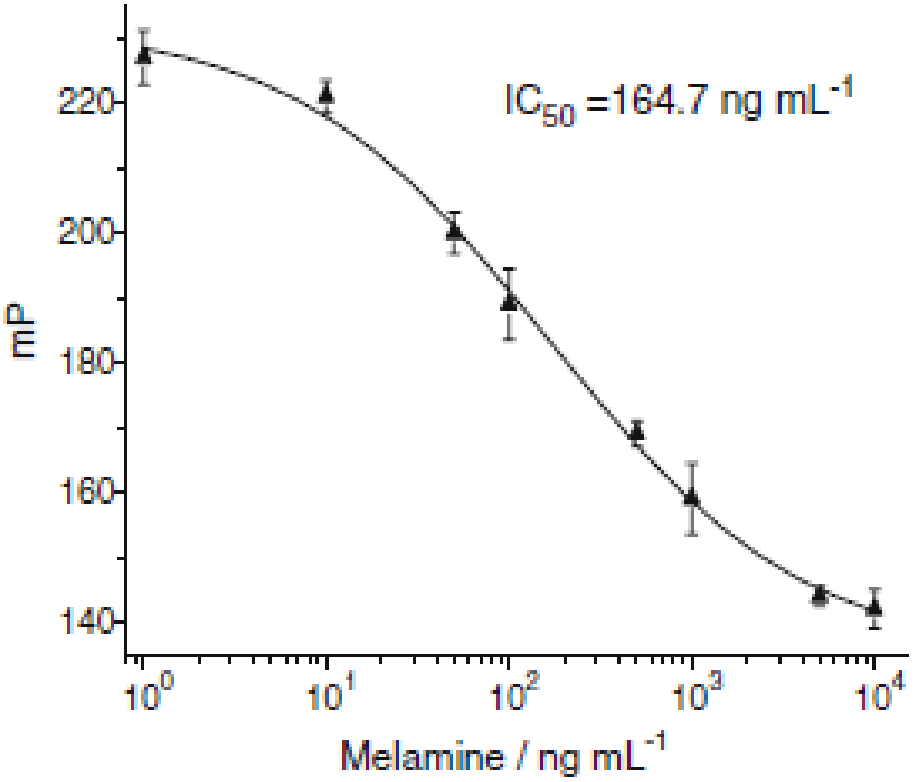


Figure 5. The standard curve of three tracers.

Qiang Wang, Simon A. Haughey, Yuan-Ming Sun, Sergei A. Eremin, Zhen-Feng Li, Hui Liu, Zhen-Lin Xu, Yu-Dong Shen, Hong-Tao Lei. Development of a fluorescence polarization immunoassay for the detection of melamine in milk and milk powder. Anal Bioanal Chem, 399(6), 2275-2284 (2011).

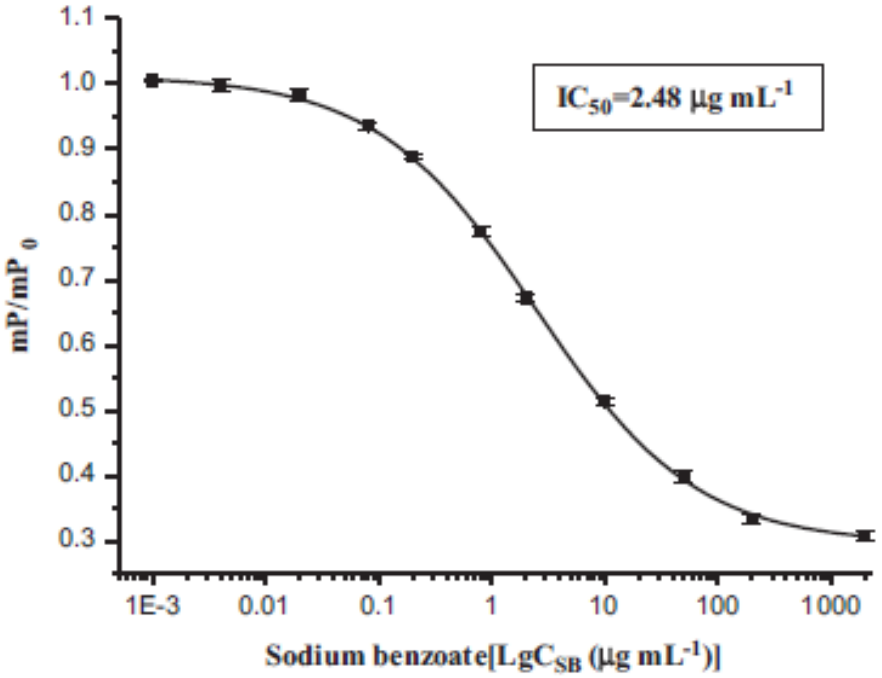


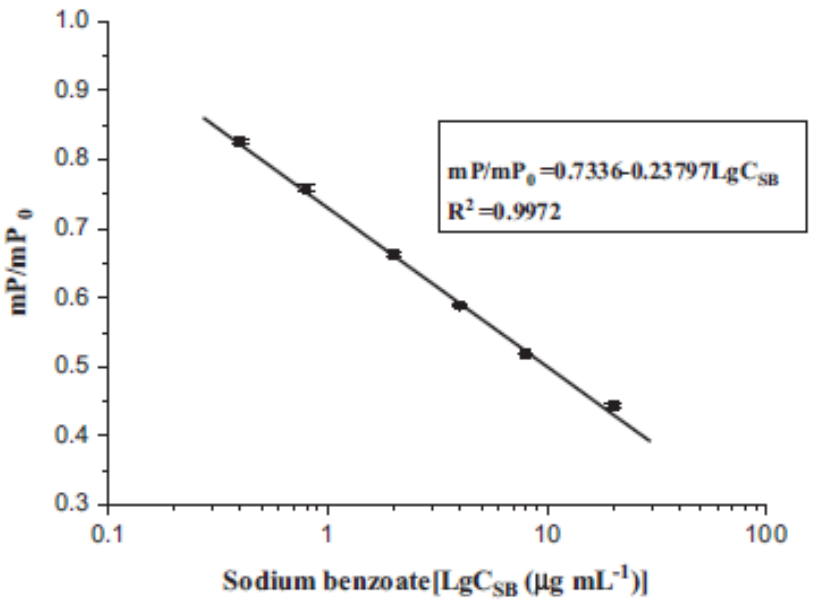


The limit of detection (10% inhibition) of the FPIA was 9.3 ng/mL

The maximum amount of melamine allowed is 1 mg kg-1 in powdered infant formula

Linlin Ren, Meng Meng, Peng Wang, Zhihuan Xu, Sergei A. Eremin, Junhong Zhao, Yongmei Yin, Rimo Xi. Determination of sodium benzoate in food products by fluorescence polarization immunoassay. Talanta, 121, 136-143 (2014).





The IC50 value for sodium benzoate - 2.48 μg/mL



Conclusion

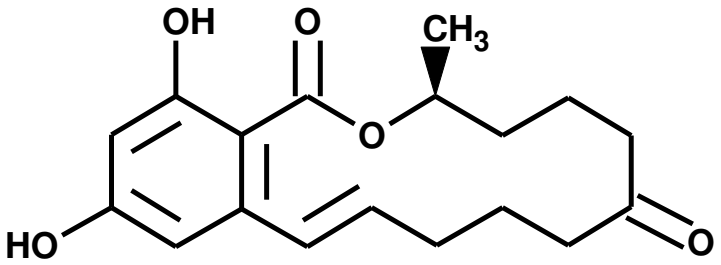
- Careful selection of immunogens for Ab production and tracers with different bridge between analyte and fluorescein could be useful for development sensitive, specific or group-specific.

Acknowledgements

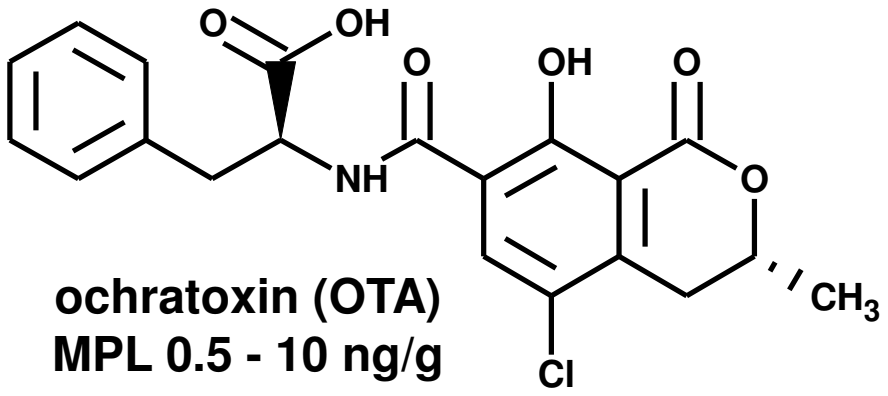
The research was supported by Grant of Russian Foundation for Basic Research (14-03-00753)


www.begann.de

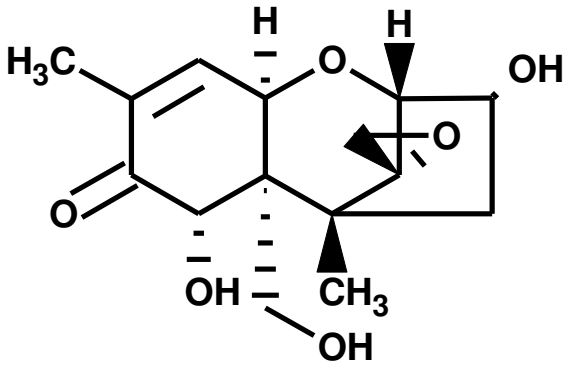
Mycotoxins in corn
(Maximum Permit Limit – MPL)



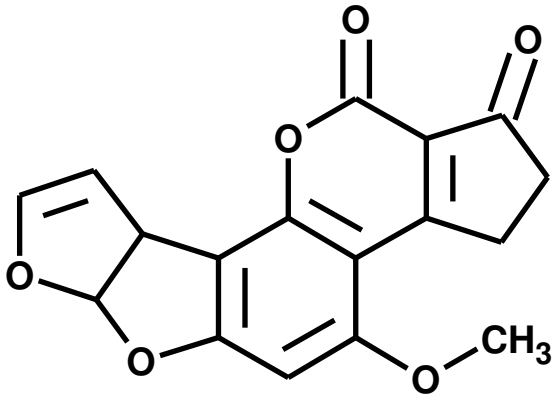
zearalenone (ZEN)
MPL 20 - 200 ng/g



ochratoxin (OTA)
MPL 0.5 - 10 ng/g

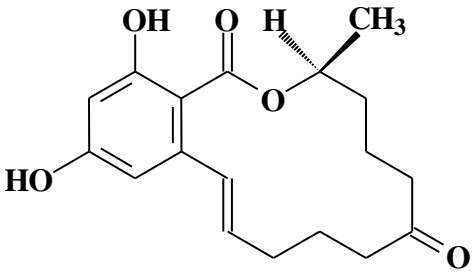


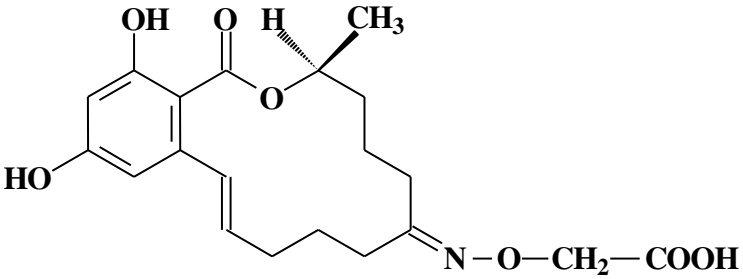
deoxynivalenol (DON)
MPL 20 - 1750 ng/g

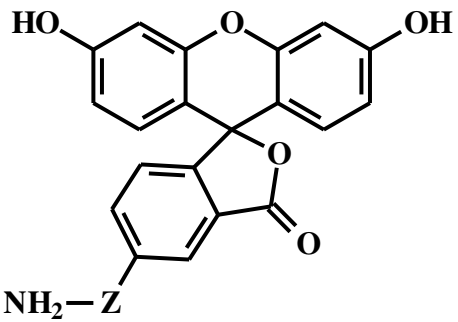


aflatoxin B1 (AFB1)
MPL 0.1 - 20 ng/g

Synthesis of fluorescein labelled antigens (tracers) for Zearalenone

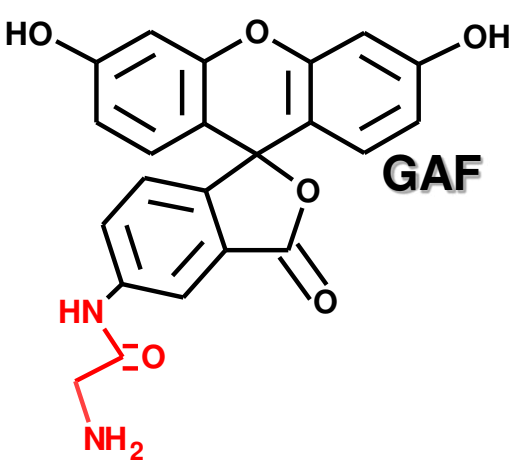




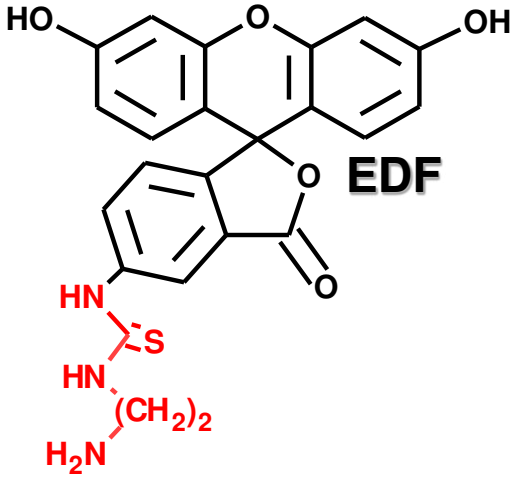


Ag-COOH + NH2-Z-Fluorescein → Ag-CO-NH-Z-Fluorescein

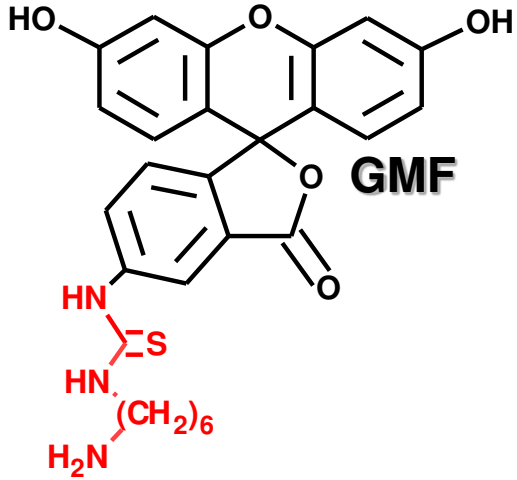
Derivatives of Fluorescein with NH2-group for ZEA-tracers



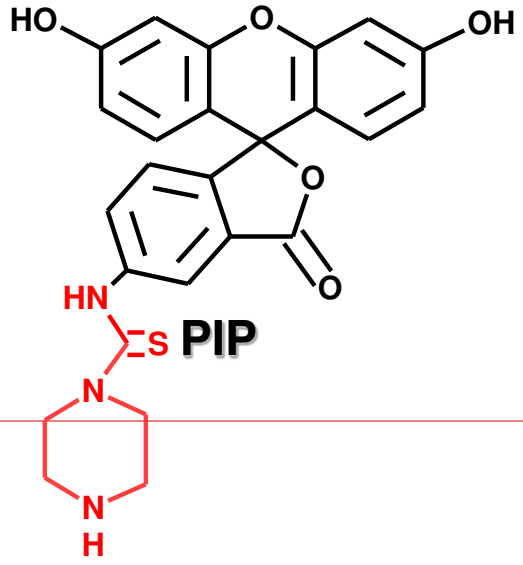
GAF



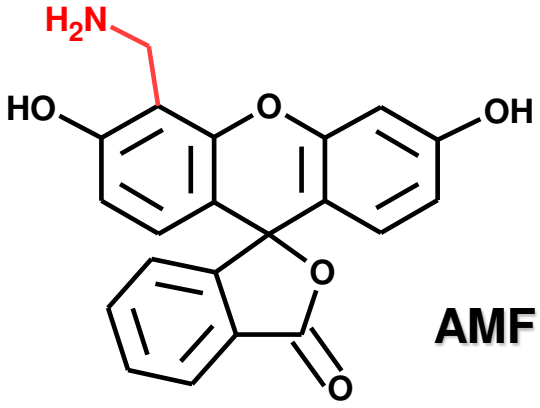
EDF



GMF

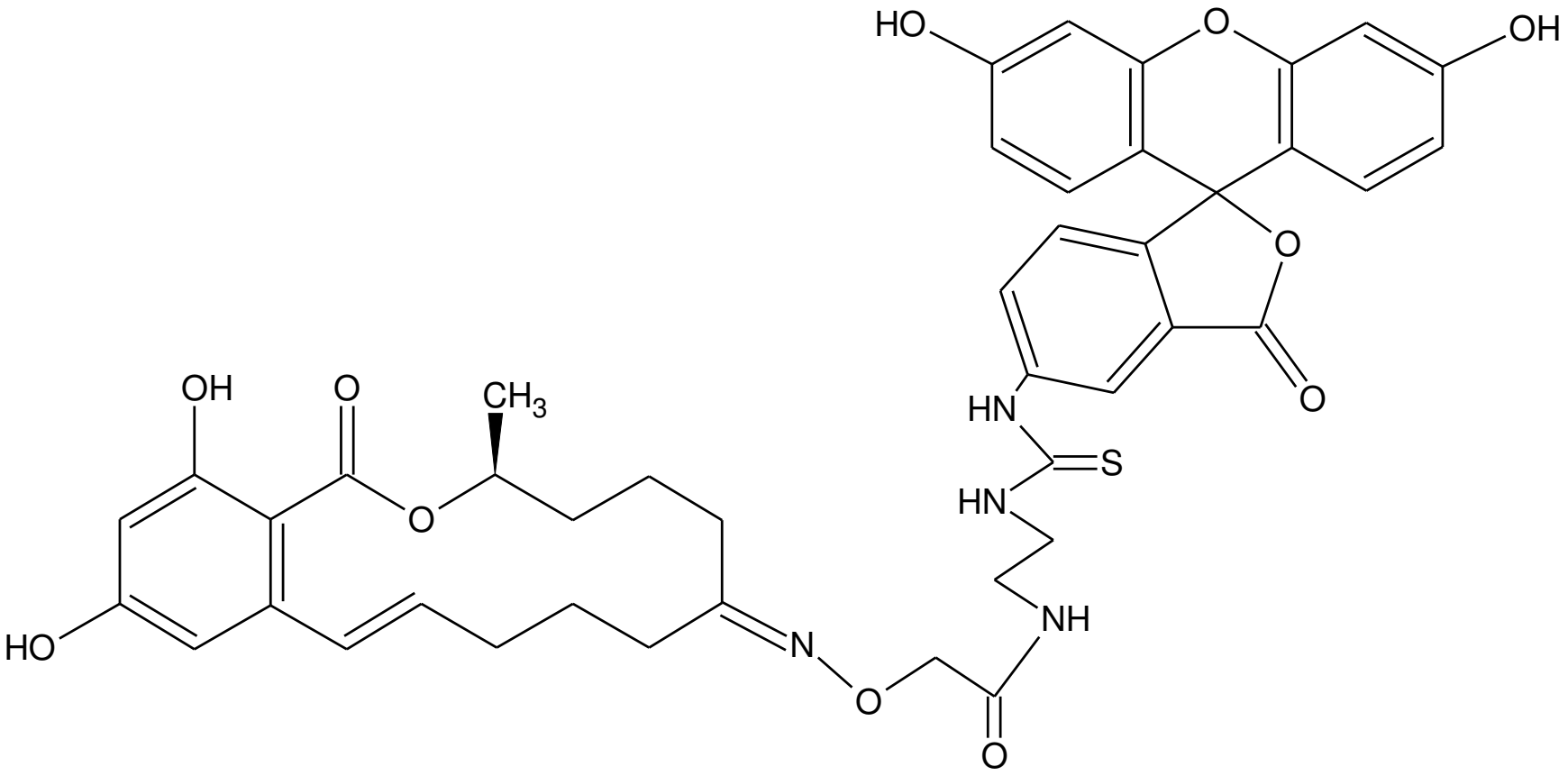


PIP

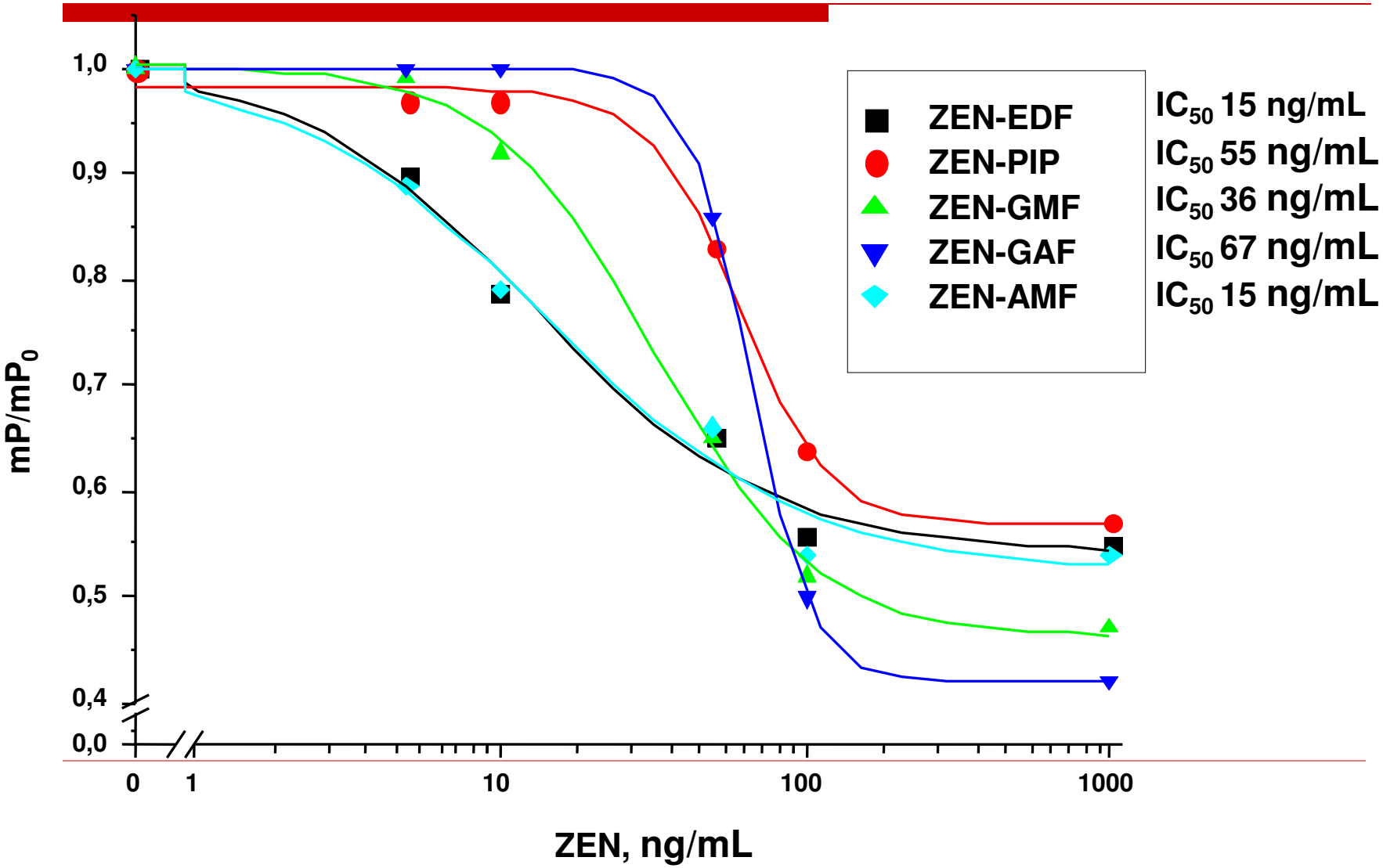


AMF

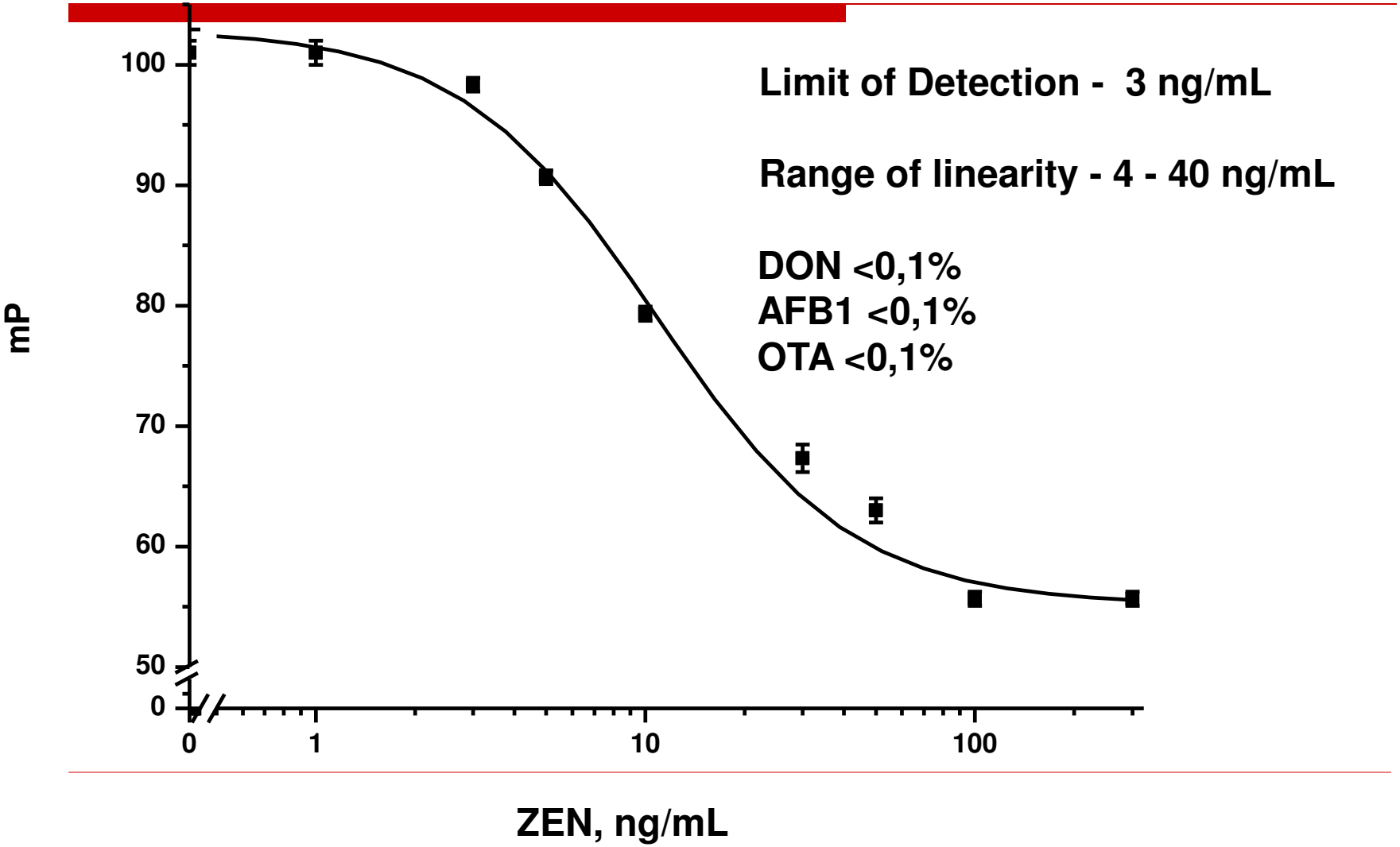
Structure of tracer for Zearalenone (ZEN-CMO-EDF)



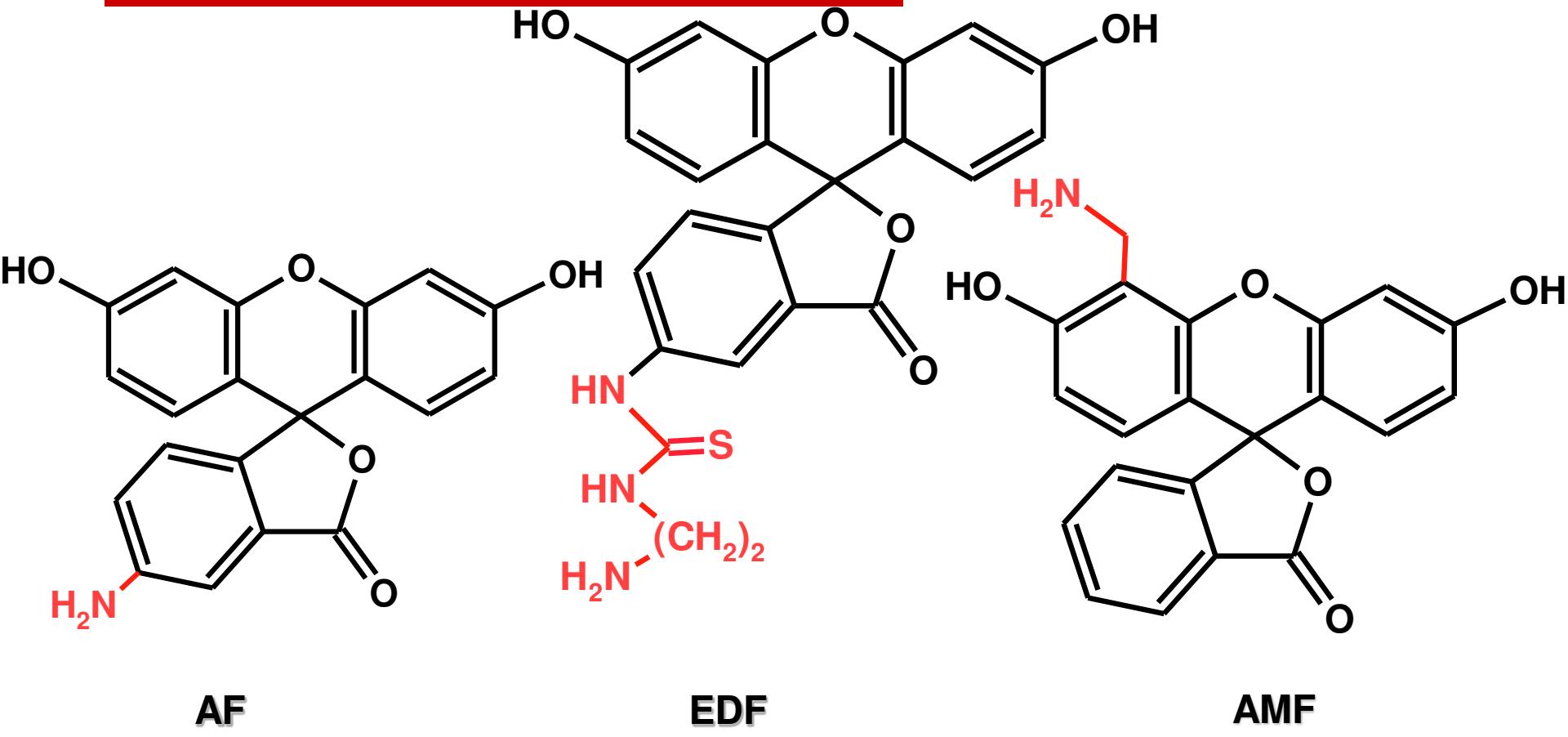
Preliminary FPIA standard curves for Zearalenone (ZEN) with different tracers



FPIA standard curve for Zearalenone (ZEN)

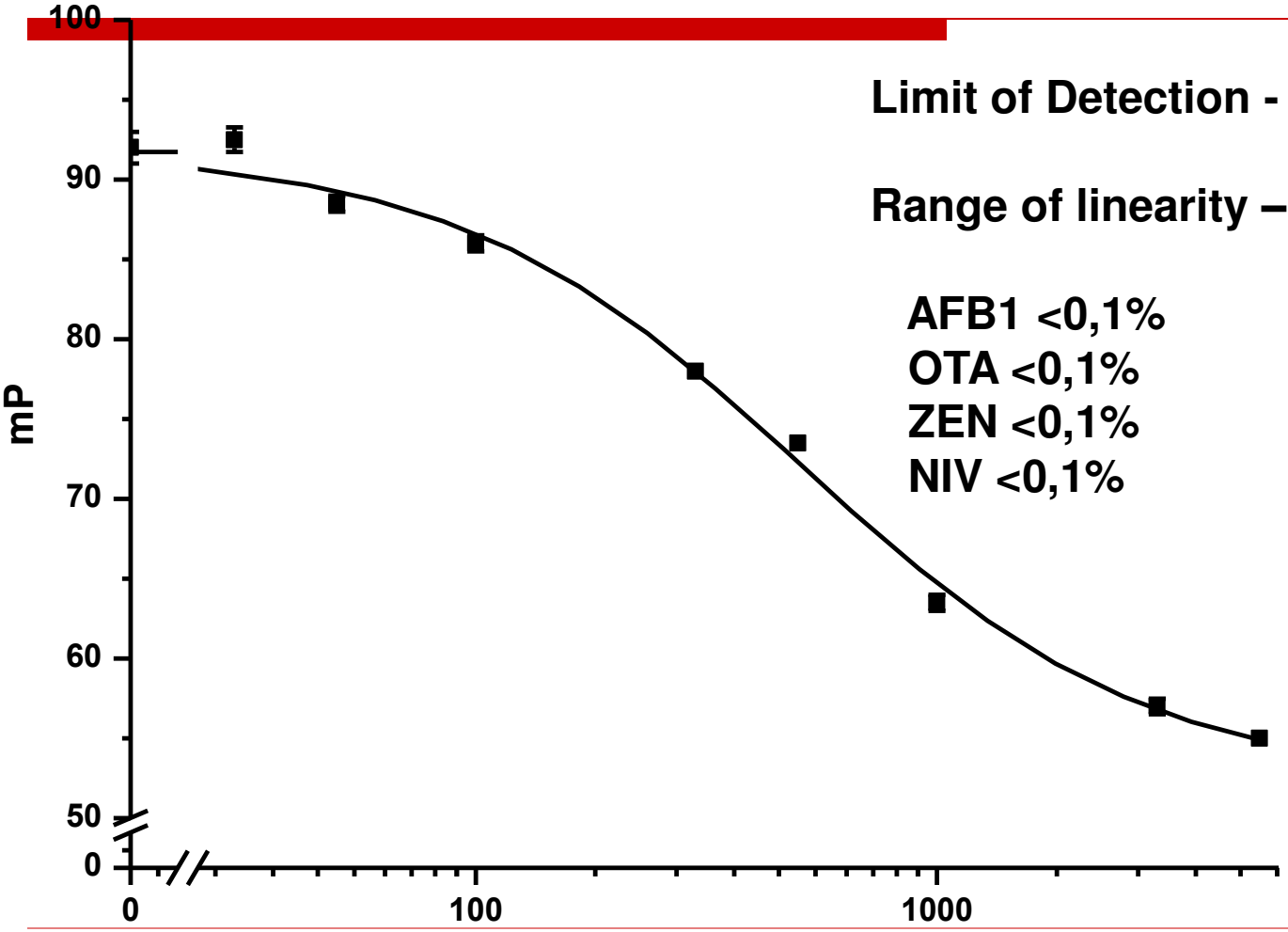


Derivatives of Fluorescein with NH2-group for DON-tracers



AF EDF AMF

FPIA standard curve for DON

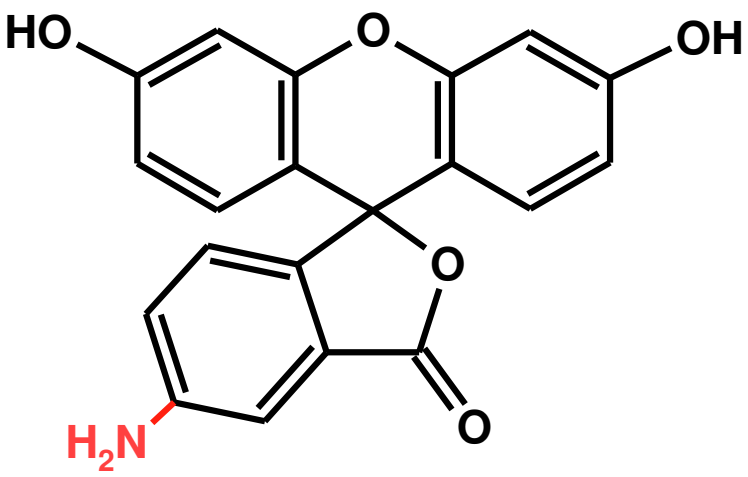


Limit of Detection - 36 ng/mL

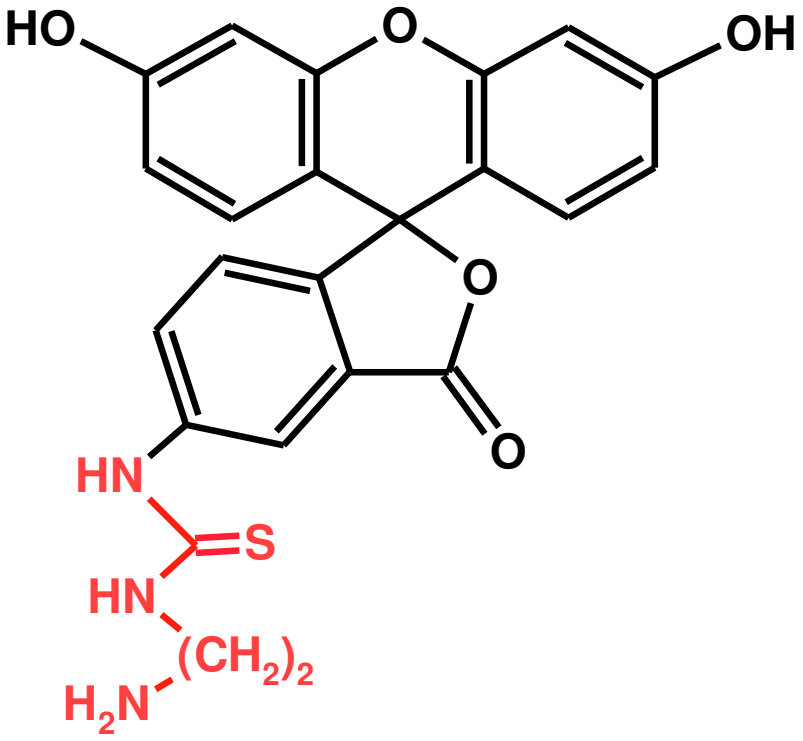
Range of linearity – 125-1000 ng/mL

AFB1 <0,1%
OTA <0,1%
ZEN <0,1%
NIV <0,1%

Derivatives of Fluorescein with NH2-group for AFB1-tracers

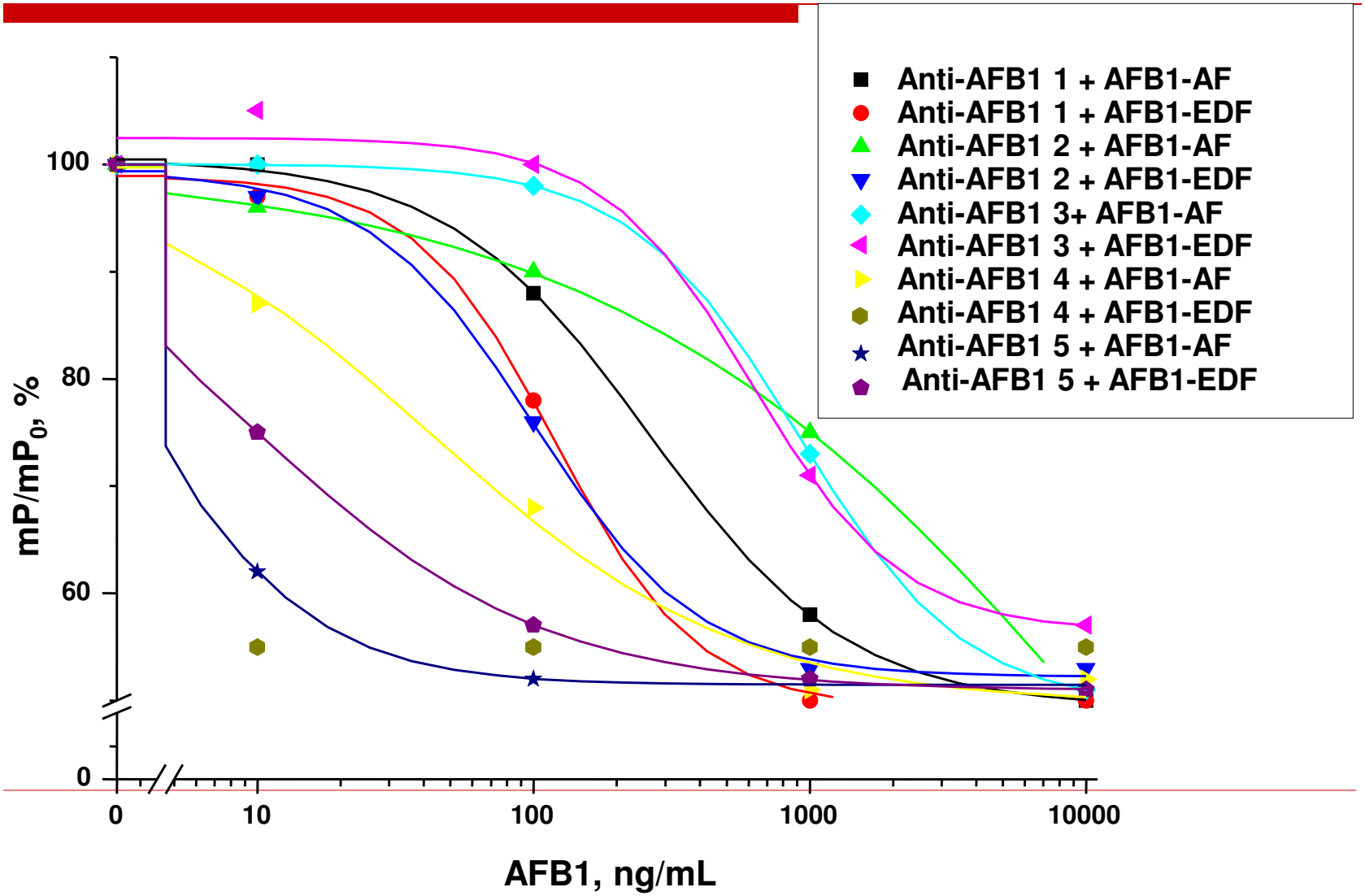


AF

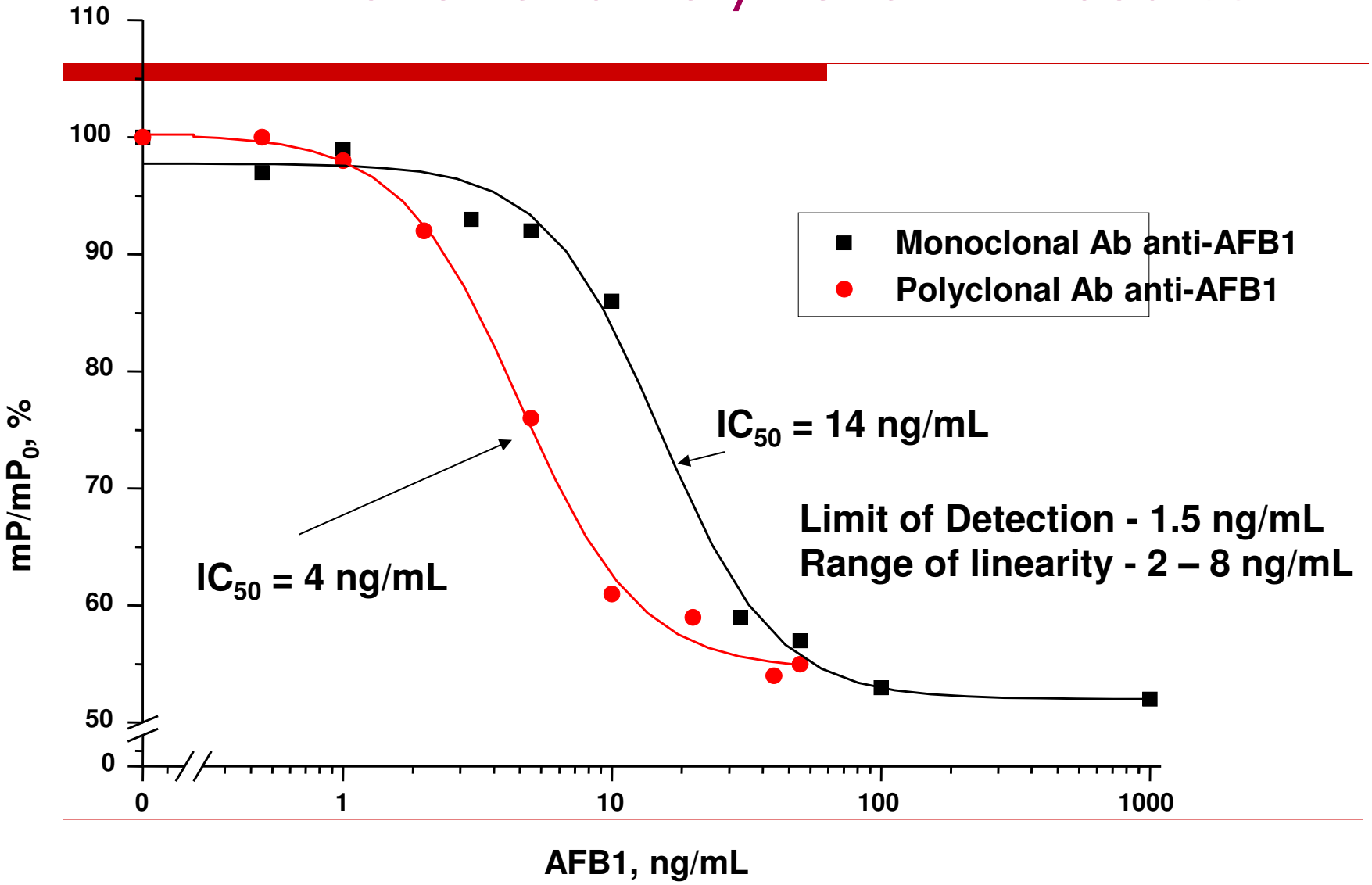


EDF

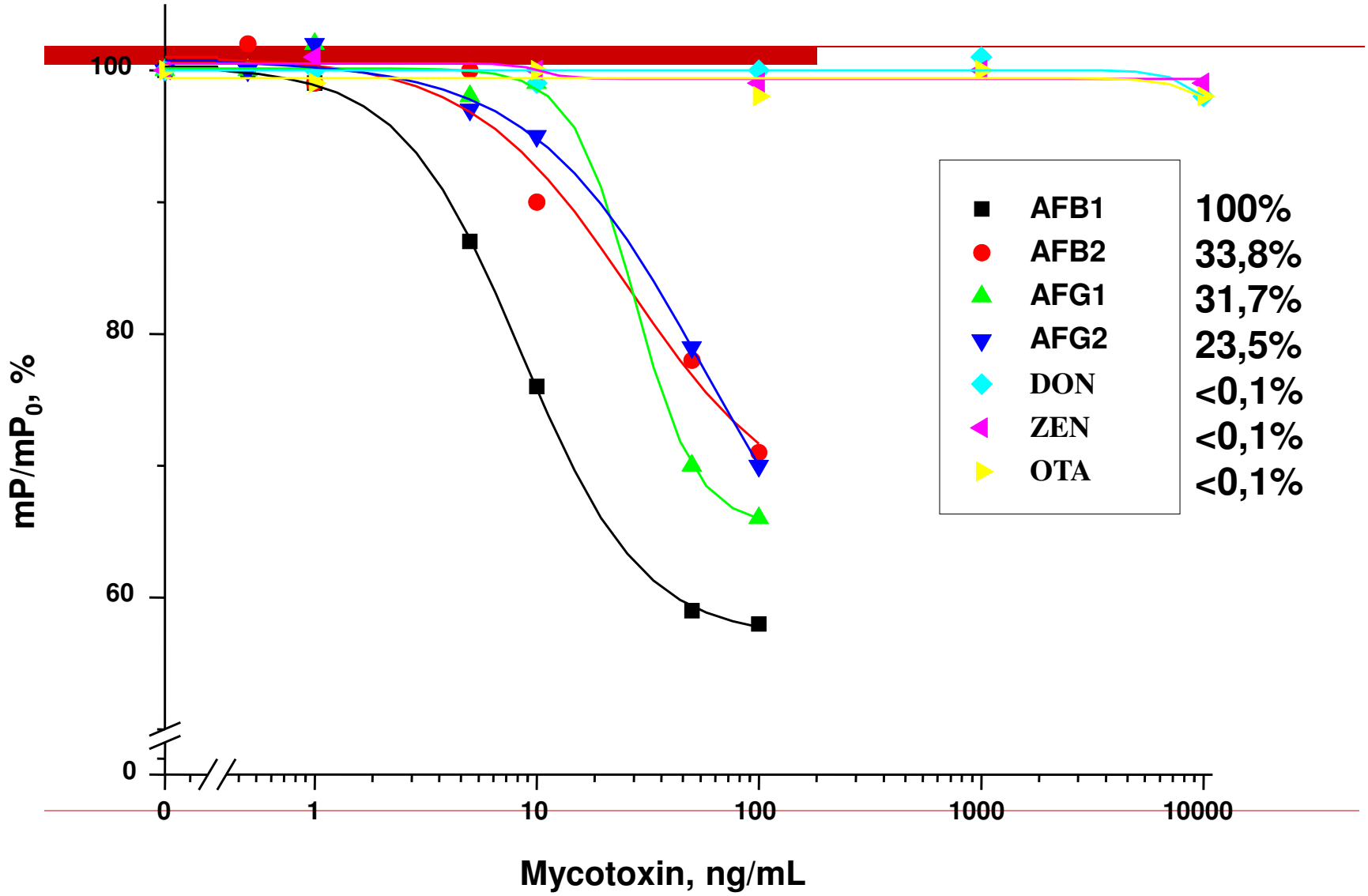
Preliminary FPIA standard curves for Aflatoxin B1 (AFB1) with 5 different Antibodies and 2 different tracers



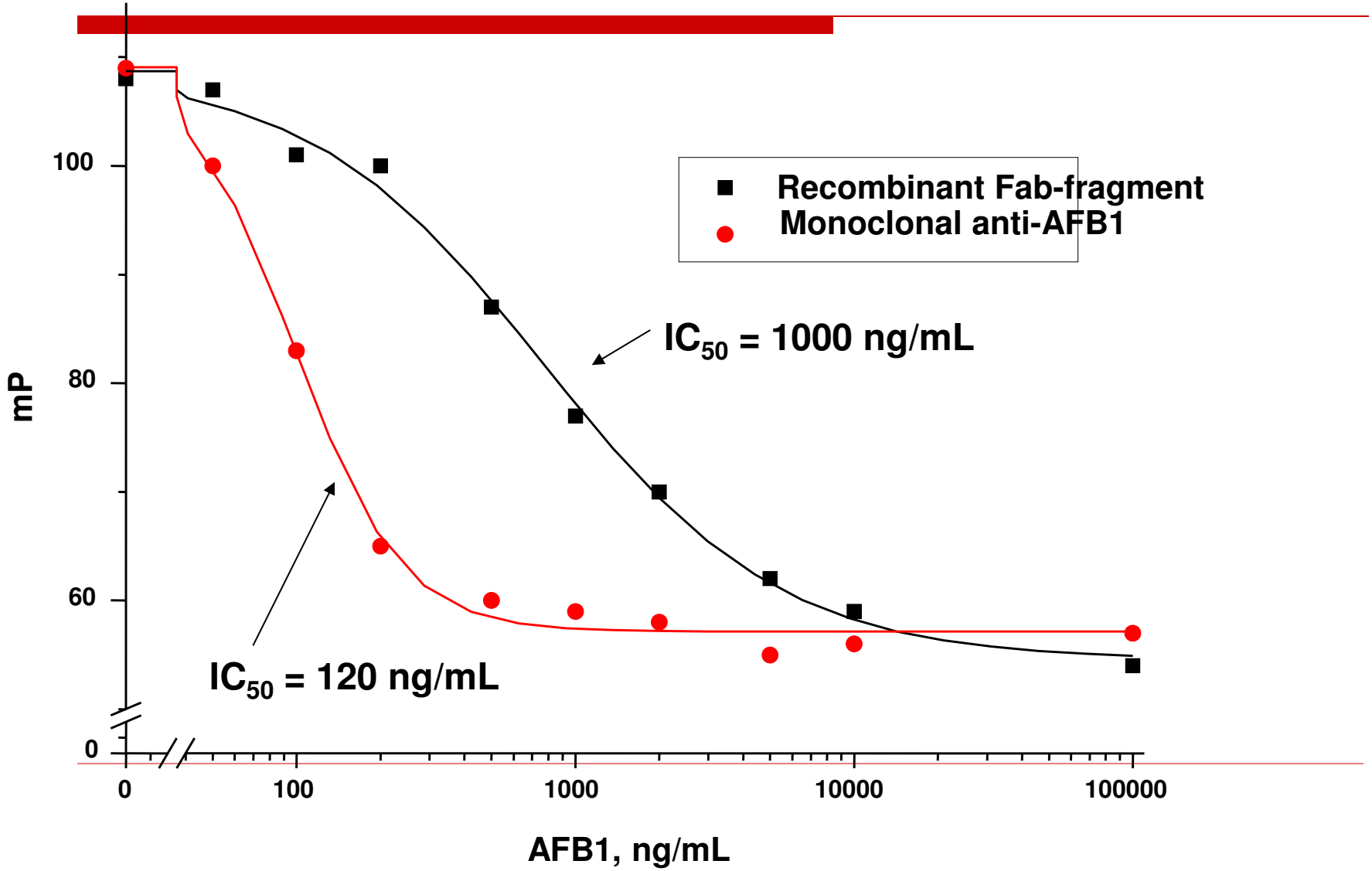
FPIA standard curves for Aflatoxin B1 (AFB1) with Mono- and Polyclonal Antibodies



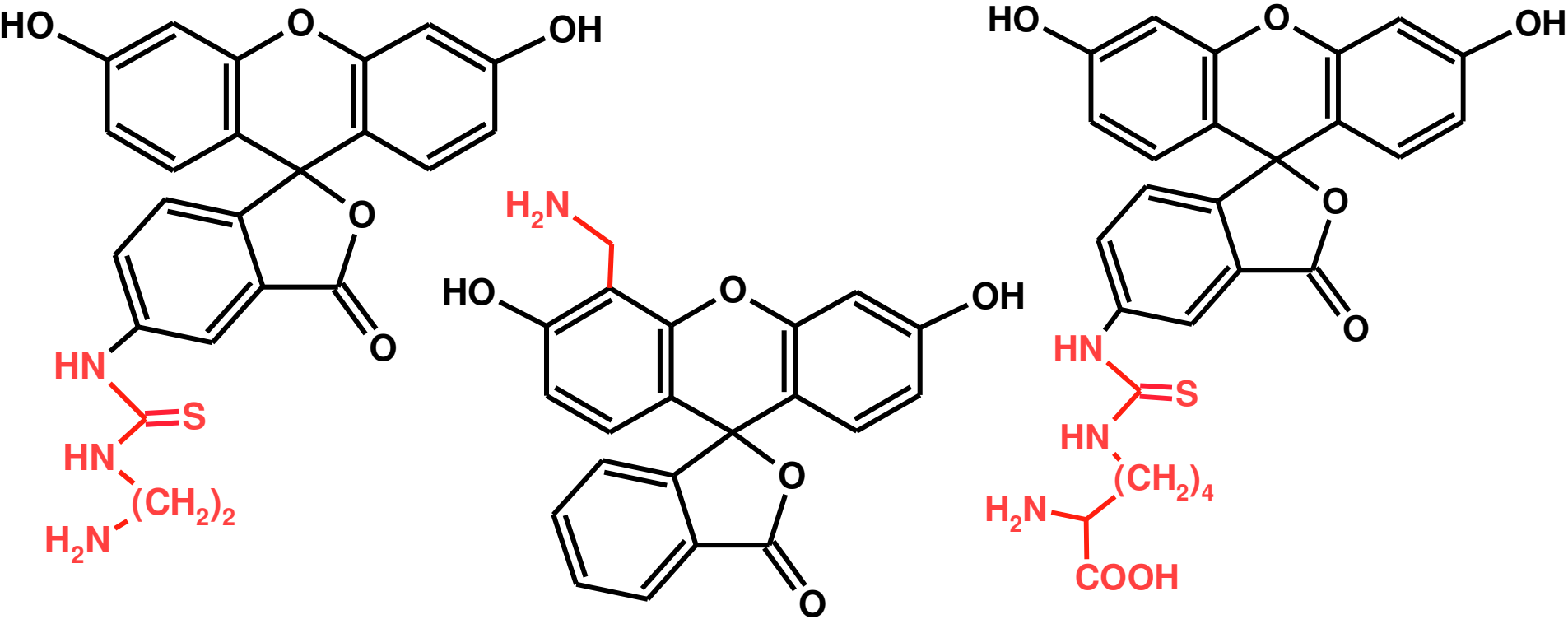
Cross-reactivity for detection of AFB1 by FPIA



FPIA standard curves for AFB1 with monAb and recombinant Fab-fragment of MonAb

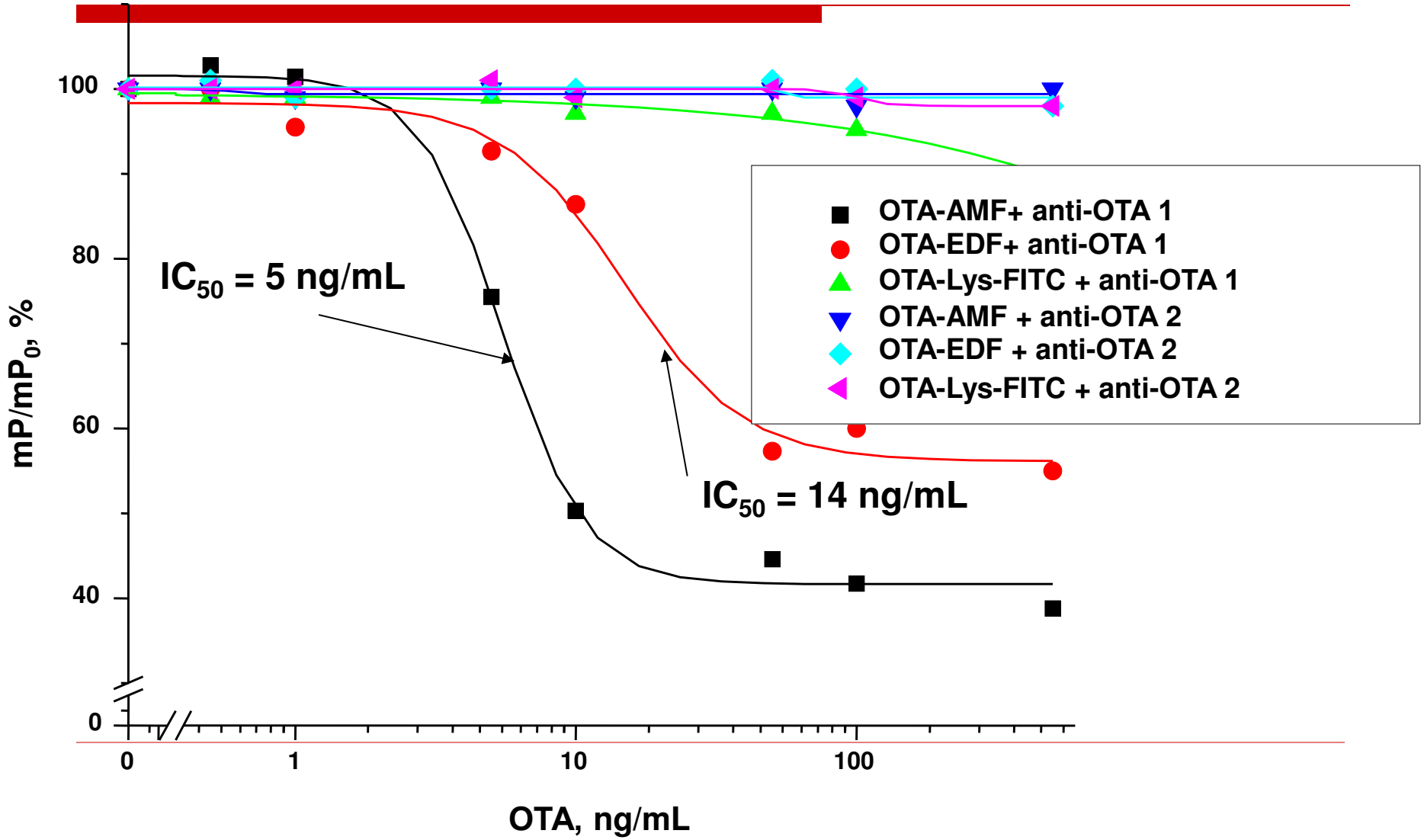


Derivatives of Fluorescein with NH2-group for OTA-tracers

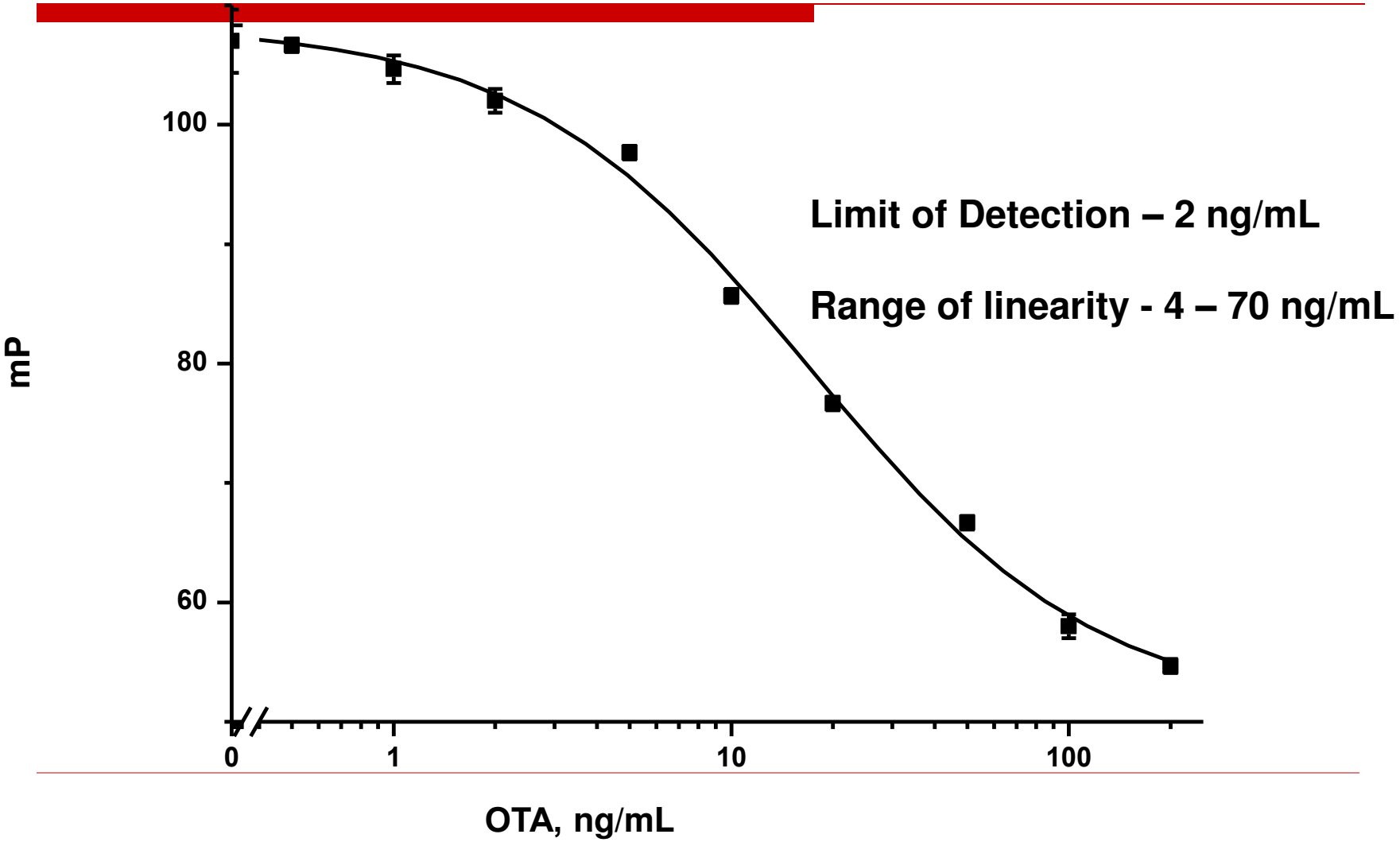


EDF AMF Lys-FITC

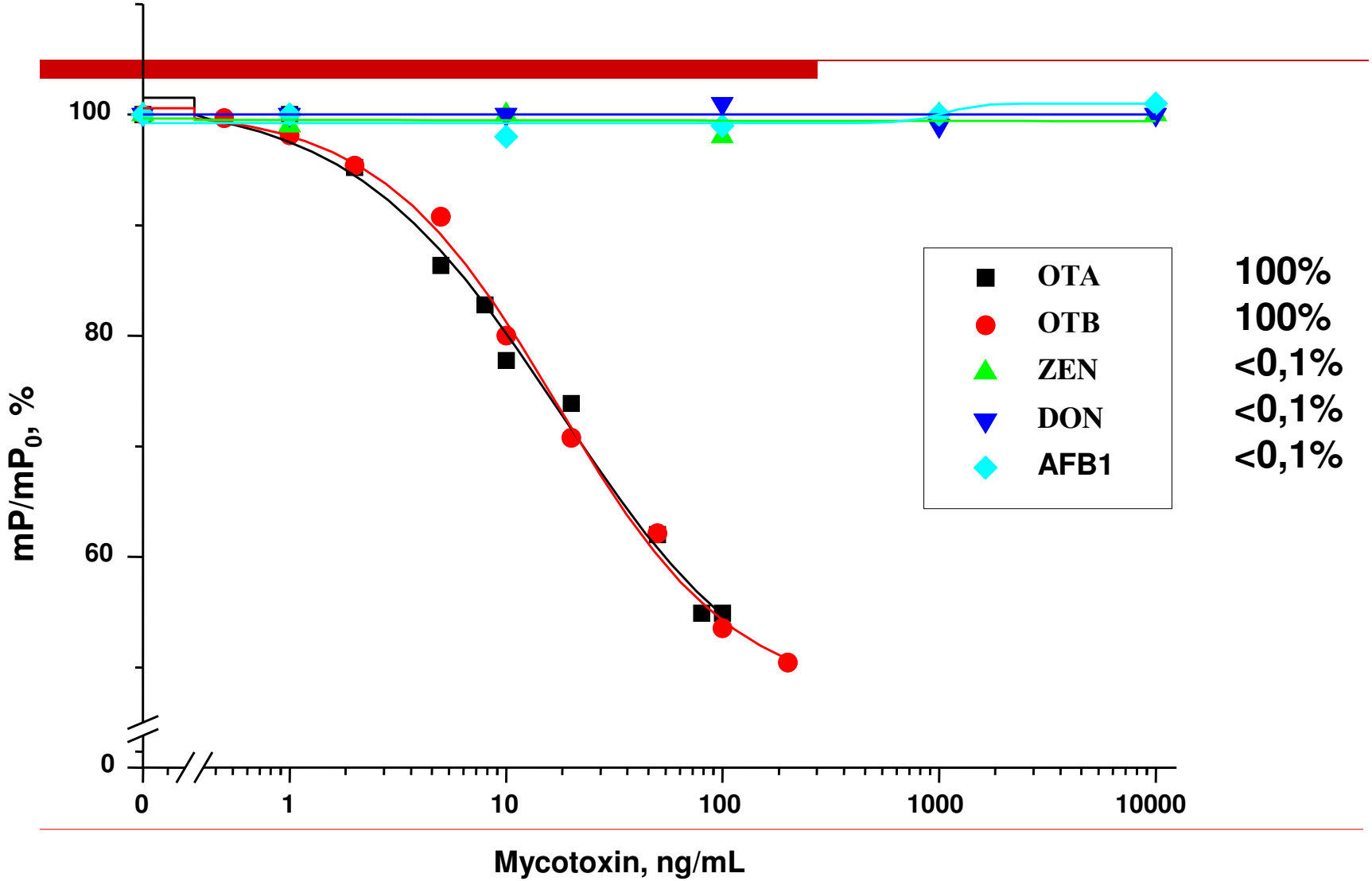
Preliminary FPIA standard curves for Ochratoxin A (OTA) with different Ab and tracers



FPIA standard curve for Ochratoxin A (OTA)



Specifisity of FPIA standard curve for Ochratoxin A (OTA)



Parameters for FPIA of mycotoxins

	Ab	Tracer	LOD, ng/mL	Range, ng/mL	MPL ng/g
ZEN	MAb	EDF	3	4-40	100
DON	MAb	AMF	36	125-1000	1250
AF	PAb	EDF	1.5	2.1-8.2	4
OTA	MAb	AMF	2	4-70	5

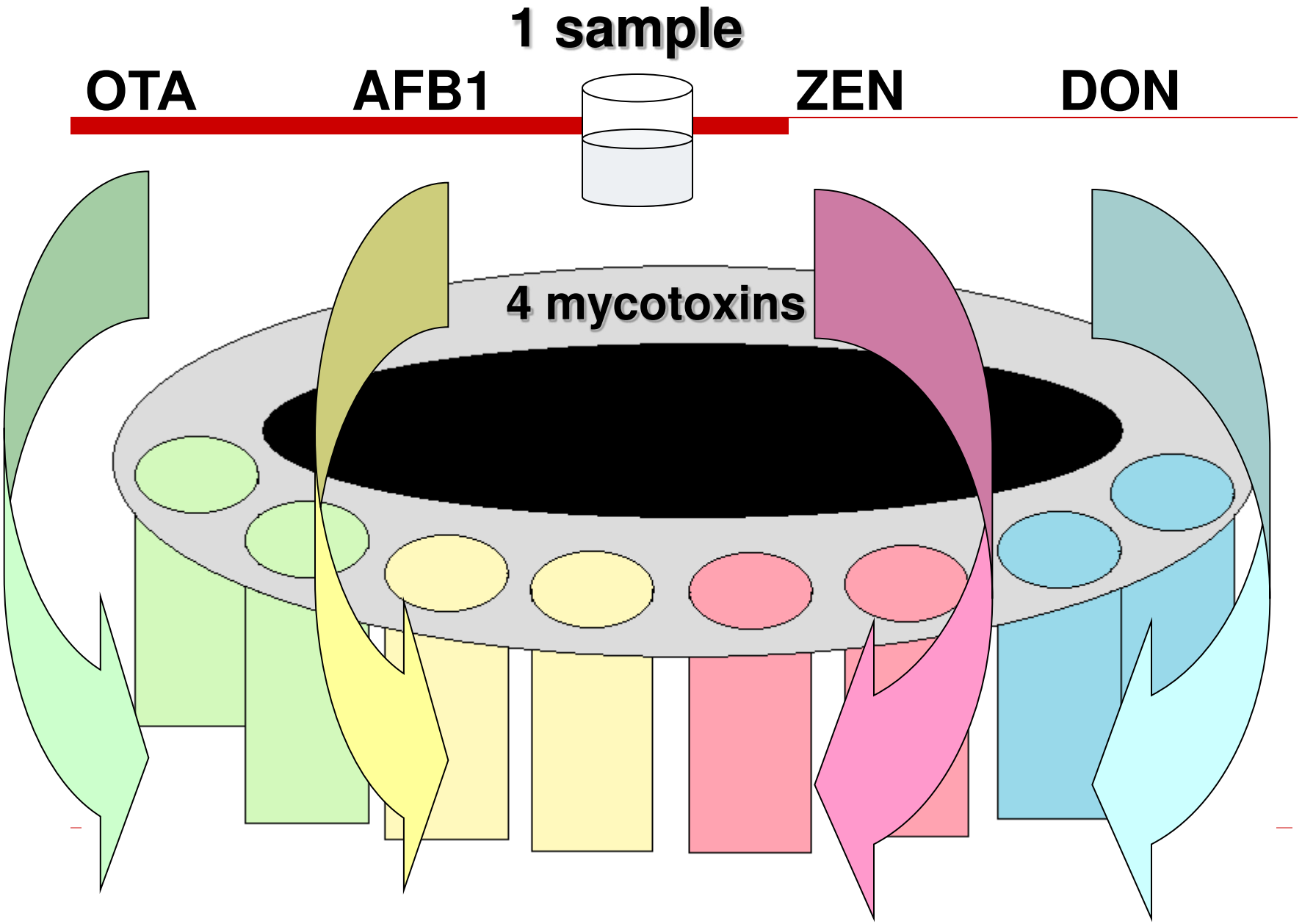


Sample pre-treatment for FPIA

- Corn sample
- 3 g corn + 15 mL eluent. Centrifugation
- Eluent: Methanol:water = 6:4
- FPIA for supernatant



Multi-FPIA



Parameters for FPIA of mycotoxins in corn samples

	Ab	Tracer	LOD, ng/mL	LOD, ng/g	Range, ng/mL	MPL ng/g
ZEN	MAb	EDF	3	15	20-200	100
DON	MAb	AMF	36	180	625-5000	1250
AF	PAb	EDF	1.5	7.5	10-40	4
OTA	MAb	AMF	2	10	20-350	5

Recovery of mycotoxins in corn samples by FPIA

	Mycotoxin	Added, ng/mL	Founded, ng/mL	Recovery, %
Spiked sample 1	ZEN	100	91±5	91
	DON	1000	960±50	96
	AFB1	20	18.5±0.9	93
	OTA	100	97±5	97
Spiked sample 2	ZEN	150	140±7	93
	DON	3000	2850±150	96
	AFB1	30	28±1	94
	OTA	300	280±10	95

Mycotoxins in corn (ng/g) detected by developed FPIA and another methods

	ZEN	DON	AFB1	OTA
Wheat 1	n.d. n.d.	n.d. n.d.	7.5±0.5 < 10	n.d. n.d.
Wheat 2	38±1 < 50	750±40 < 1000 нг/г	n.d. n.d.	n.d. n.d.
Wheat 3	n.d.	280±20	n.d.	n.d.
Wheat 4	n.d.	220±10	n.d.	n.d.
Wheat 5	n.d. n.d.	n.d. n.d.	13±1 17.5±2	n.d. n.d.
Wheat 6	100±5 104.4±1	n.d. n.d.	n.d. n.d.	n.d. n.d.
Rice	n.d. n.d.	n.d. n.d.	n.d. n.d.	n.d. n.d.



Principe of Fluorescence Polarization ImmunoAssay

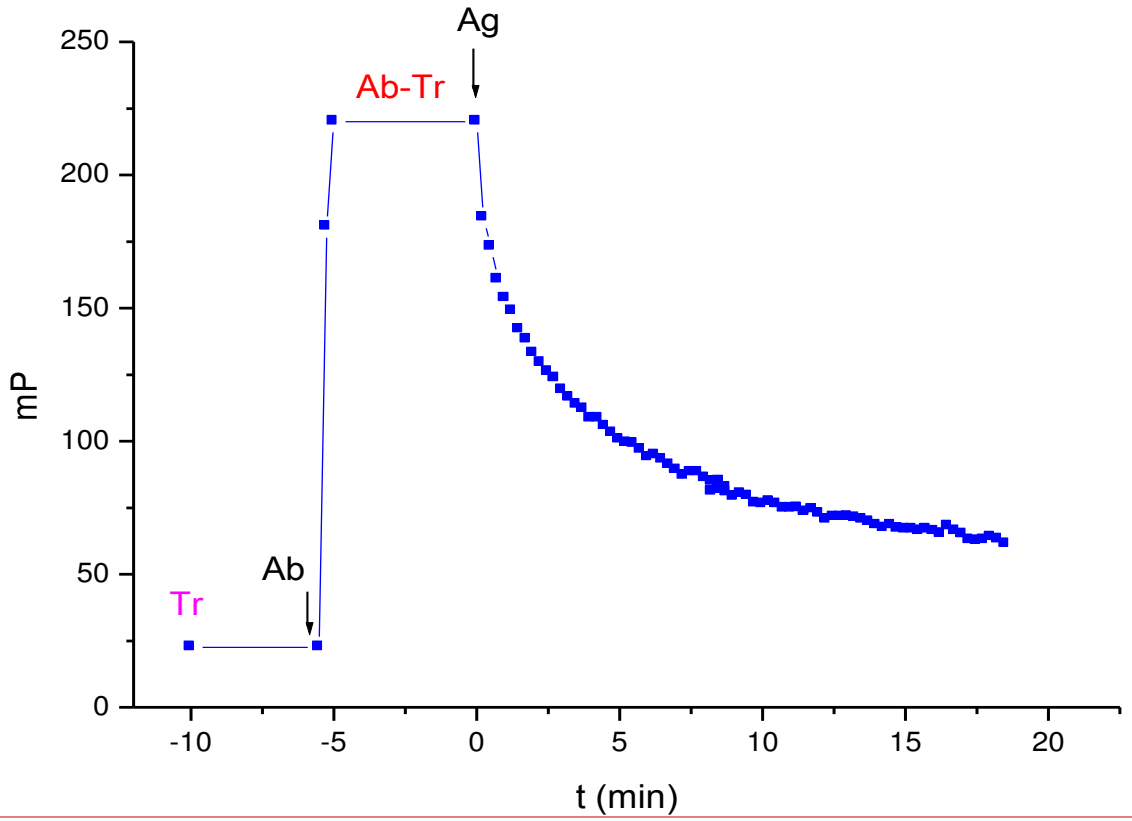
Standard FPIA

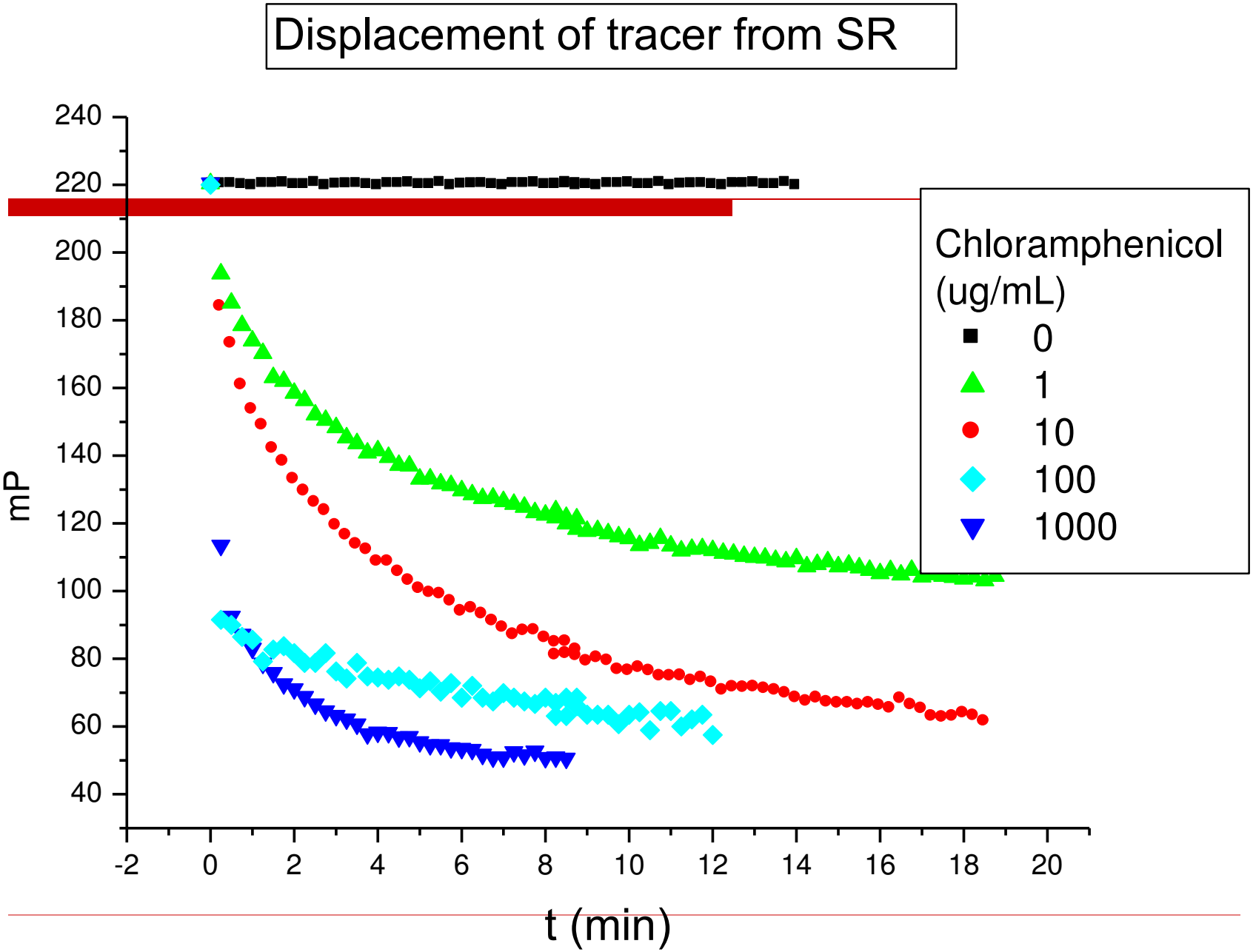


Single-Reagent FPIA

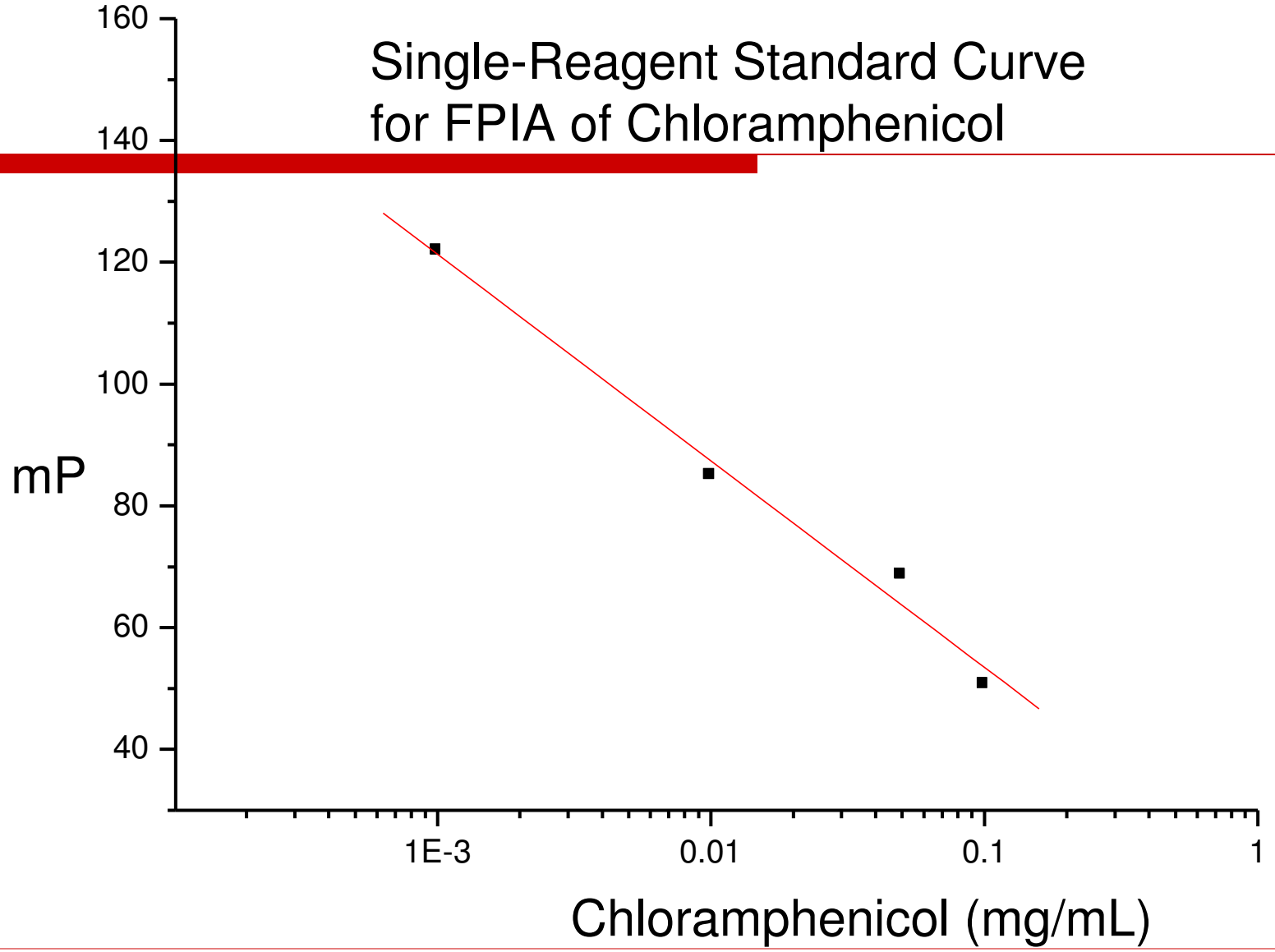


Kinetic of association (Ag* + Ab = Ag*:Ab) and dissociation of immuno-complex (Ag*:Ab + Ag = Ag:Ab + Ag*) for Chloramphenicol




Displacement of tracer from SR
240
220
200
180
160
140
120
100
80
60
40
mP
-2
0
2
4
6
8
10
12
14
16
18
20
t (min)
Chloramphenicol (ug/mL)
0
1
10
100
1000

Single-Reagent Standard Curve for FPIA of Chloramphenicol

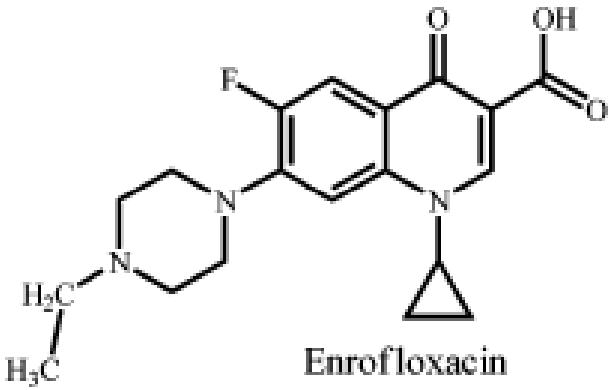


Thanks for attention



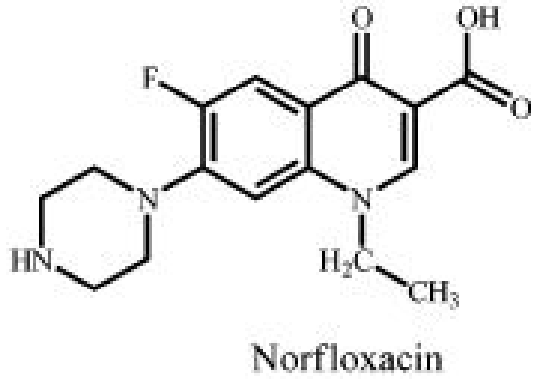
Fluoroquinolones

Enrofloxacin (ENR) Norfloxacin (NOR)
Ofloxacin (OFL) Ciprofloxacin (CIP)



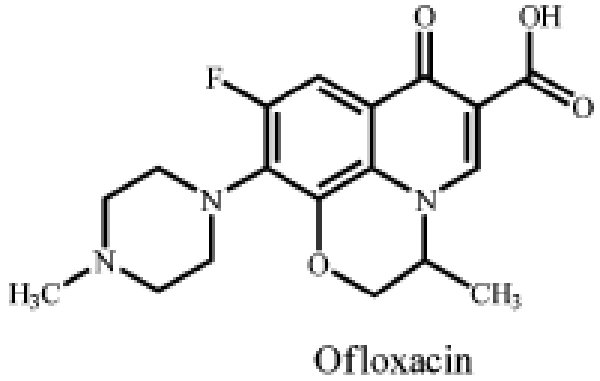
Enrofloxacin

Enrofloxacin (ENR)



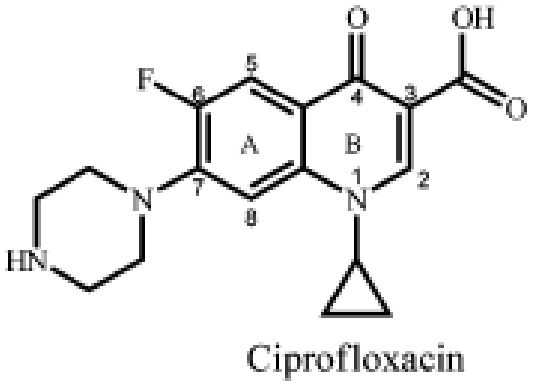
Norfloxacin

Norfloxacin (NOR)



Ofloxacin

Ofloxacin (OFL)

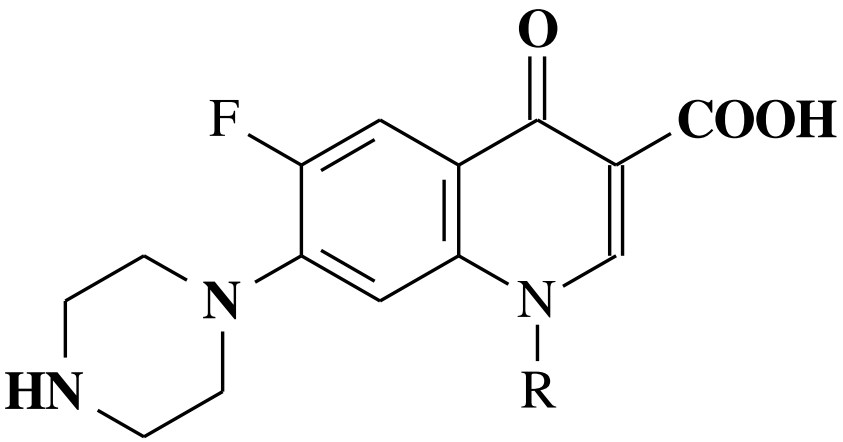


Ciprofloxacin

ciprofloxacin (CIP)

Specific and group-Specific detection

Specific and group-specific immunoassays for FQ



Specific

Group-specific