Single-molecule fluorescence system for determination of biogenic amines in biological samples

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**Biogenic amines**

- **Matrix of hydrogel**
- **Fluorescent system**
- **Immobilization of the system**
- **Toxicity for cells**
- **"sandwich" system**

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**Neurotransmitters**

Biomarkers of neuroendocrine and neurodegenerative diseases

- Adrenaline
- Noradrenaline
- Dopamine
- Serotonin

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**The Goal**

Early diagnosis of neurodegenerative diseases can be based on the determination of decreased content of biogenic amines in cell cultures.

Immobilization of the fluorescent system for biogenic amines (BA) determination in hydrogels matrixes as the part of detecting system and the medium for cells growing.
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**NATURAL HYDROGELS**

- Ex vivo mammalian cell cultivation for studying the physiology of cells and tissues, to diagnose and predict diseases.
- 3D growing imitating natural cells of living organisms

- Rigid 3D matrices
- Increasing the quantum yield of fluorescence by complex stabilizing

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**Cell cultures growing medium**

- Cell cultures growing medium

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**Fluorescent systems immobilization matrixes**
Biogenic amines (BA) determination by the formation of highly fluorescent triple complexes Eu\(^{3+}\)-OTC-BA in micellar solution in the single molecular level.
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Alginate hydrogel
Collagen hydrogel

Good results

Conc. range, pM
LOQ, pM
S_r (n = 4, P = 0.95)

500 - 5000
300
0.008

5 - 50
2
0.007

5 - 50
3
0.009

Very dense

Conc. range, pM
LOQ, pM
S_r (n = 4, P = 0.95)

500 - 5000
210
0.010

100 - 1000
60
0.030

50 - 500
25
0.044

100 - 1000
70
0.037

Dopamine
Adrenaline
Noradrenaline

Bad results

Gelatin hydrogel
Albumin hydrogel

Conc. range, pM
LOQ, pM
S_r (n = 4, P = 0.95)

500 - 5000
280
0.006

100 - 1000
65
0.009

5 - 50
3
0.011

Alginate hydrogel
Collagen hydrogel

✓ Good reproducibility
✓ Highest compatibility with living cellular systems

Idea: to combine these two advantages
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PC 12 cells are cloned from pheochromocytoma, a tumor of the chromaffin tissue of the adrenal glands, and are similar in their ability to synthesize, accumulate and excrete neurotransmitters with chromaffin cells.

The toxicity study of the components of the indicator reaction (Eu³⁺, OTC, MOPS, TWEEN 80 and Eu³⁺ + OTC + MOPS) for the viability of PC12 cells.

Determination of the cell viability using the fluorometric method «CellTiter-Blue® Cell Viability Assay (Promega)»
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Analyte | Conc. range, pM | LOQ, pM | \( S_r \) (n = 4, P = 0.95)
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**Alginate hydrogel**
Dopamine | 500 – 5000 | 300 | 0.008
Adrenaline | 5 – 50 | 2 | 0.007
Noradrenaline | 5 - 50 | 3 | 0.009

**Alginate-collagen sandwich**
Dopamine | 90 – 400 | 83 | 0.013
Adrenaline | 2.5 – 15 | 1.0 | 0.008
Noradrenaline | 2 – 25 | 0.8 | 0.030

Spatial separation of toxic components of the reaction and living cell cultures
Reducing the detection limits

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