

New GABA ELISA based on an unique high throughput extraction procedure

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Abstract

Introduction: The new GABA (γ -aminobutyric acid) ELISA is especially designed to measure GABA in different biological fluids (eg. brain and spinal cord homogenates, cell culture). With this new assay improved sensitivity is achieved and less sample volume can be used. To achieve all this a new extraction procedure was developed. Instead of using the laborious ion exchange column procedure, the ion exchange resin was coated to a microtiter plate (patent pending). It showed that after the coating, the ion exchange kept its unique characteristics. The advantage of using an extraction plate instead of a column: no "dead" volume, faster throughput, higher sensitivity. For the subsequent ELISA procedure a highly specific GABA antibody is used. A proof of principle was performed with rat brain and spinal cord tissue homogenates. Samples of rat brain and spinal cord were homogenized and spiked with known concentrations of GABA and recovery and linearity of the GABA ELISA was tested.

Results: GABA standards were treated like samples and corresponded to 250 – 25000 pg/well. Only 10 μ L homogenate was needed for the analysis. Recovery from brain homogenate (cortex) was 83 – 100 %, for spinal cord homogenate (lumbar region) 85 – 92 %. Linearity for both samples was 98 – 115 %. Different brain and spinal cord regions were analyzed (brain: cortex and hippocampus, spinal cord: cervical, thoracic and lumbar region). Results were in line with previous HPLC results on the same kind of samples.

Conclusion: A sensitive GABA ELISA with a new extraction procedure was developed. Based on this straightforward method high throughput testing of samples is possible. The results show that it is possible to measure GABA in different kind of homogenates.

Extraction

Sample preparation (flexible volumes)

1. GABA is extracted by ion exchange chromatography. Microtiter plates (48 Well) are coated with a cation exchange resin (patent pending).
2. After washing away unbound molecules, the bound GABA is released. Whole extraction procedure takes 30 minutes only.
3. In a simple derivatization step GABA is coupled to a protein. This is needed for the ELISA

Samples:

- brain homogenates
- spinal cord homogenates
- cell culture supernatants

Extraction plate



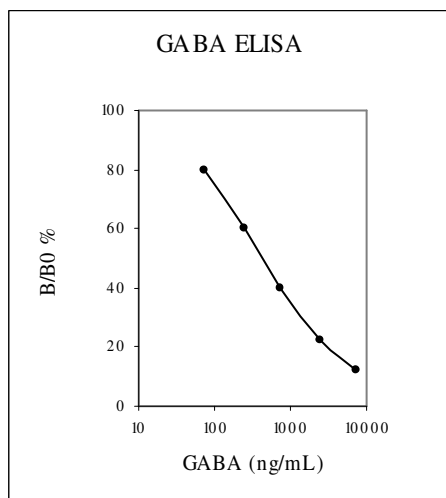
Immunoassay

1. 50 μ L derivatized sample
2. 50 μ L specific antiserum
Incubation overnight at 4 – 8°C
3. Three wash cycles with 300 μ L wash buffer
4. 100 μ L Enzyme conjugate
Incubation for 30min at RT (shaker)
5. Three wash cycles with 300 μ L wash buffer
6. 100 μ L Substrate
Incubation for 20-30min at RT (shaker)
7. 100 μ L Stop solution
Measure at 450nm



Validation

Typical standard curve



Assay characteristics

Standard Range 25 – 2500 or 75 – 7500 ng/mL
(depends on applied sample volume)
Sample volume 10 – 100 & 300 μ L
Detection limit 30 ng/mL

Results recovery & linearity

Sample	Recovery
Motor cortex	Mean recovery: 88 % SD=7.9; CV=9.0 R ² =0.999
Spinal cord Lumbar region	Mean recovery: 87 % SD=3.6; CV=4.1 R ² =0.997
Sample	Linearity
Hippocampus	Mean linearity: 105 % SD=8.2; CV=7.8 R ² =0.997

Measured rat samples*

Tissue sample	Measured conc. (pmol/mg wet weight tissue)
Motor cortex	1137 ± 65
Somatosensory cortex	1379 ± 115
Hippocampus	1719 ± 219
Cervical sc region	484 ± 65
Thoracic sc region	395 ± 19
Lumbar I & II sc region	540 ± 80
Lumbar III – VI sc region	737 ± 32
Sacral sc region	687 ± 48

*Samples were kindly provided by E. Ziemińska, Nencki Institute, Poland

