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Development of a novel highly sensitive oxytocin ELISA

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Oxytocin ELISA kit *Wako*

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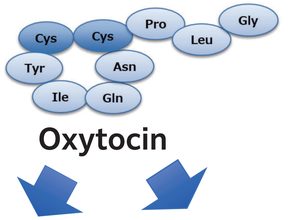
Background

Oxytocin is a peptide hormone comprising nine amino acids. It is produced in the hypothalamus and released mainly from the posterior pituitary gland following childbirth and during lactation, and promotes uterine contraction and milk production. It also has stress-relieving, anxiolytic, and fear-reducing effects and plays a role in the development of maternal behavior. Due to these properties, oxytocin is often referred to as the "happy hormone" or "love hormone." Recently, oxytocin has attracted attention in treating mental disorders such as depression and autism and in the development of functional food materials.

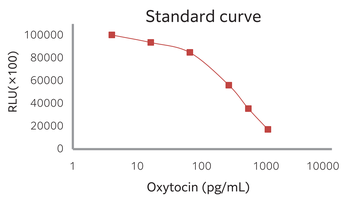


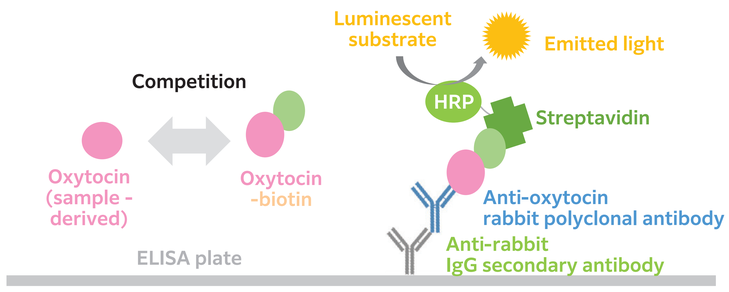
Commercially available ELISA kits require a large volume of sample and complicated pretreatment using a C18 column and organic solvent.

We developed a novel ELISA for oxytocin needing only simple pretreatment and a small sample.

Assay principal

We constructed an oxytocin competition ELISA with high sensitivity (4 pg/mL) using biotin-labeled, streptavidin-conjugated HRP and luminescent substrate.





Sample pretreatment

Oxytocin binds to proteins and other molecules in the sample, and these can affect measurement by inhibiting oxytocin-antibody interaction (i.e., interactions between oxytocin and antibodies). We investigated various methods for removing the impurities in the sample and found the following novel pretreatment method without extraction process using organic solvent.

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Sample (saliva, serum, plasma, urine)
↓
Add sample buffer 1 (acid)
↓
Mix, and incubate 5 min at R.T. , and mix incubate 5 min at R.T.
↓
Add sample buffer 2 (gel)
↓
Mix, and incubate 5 min at R.T., and mix incubate 5 min at R.T.
↓
Centrifuge
↓
Use the supernatant as a sample for measurement.

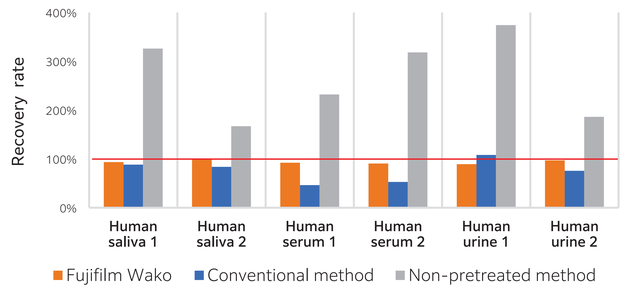
Conventional method

Sample (saliva, serum, plasma, urine)
↓
Add TFA, and mix
↓
Centrifuge
↓
Add the supernatant to C18 Column equilibrated with TFA and acetonitrile
↓
Wash with TFA, and elute with acetonitrile
↓
Depressurize and dry
↓
Dissolve and use the solution as a sample

	Fujifilm Wako	Conventional method
Preparation time	30 min	2 h-overnight
Sample volume	50-200 μL	250 μL-3 mL
Recovery rate	90-120%	42-110%

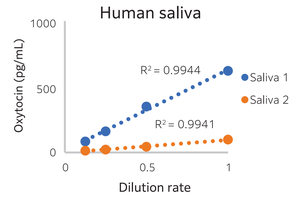
Spiked-recovery test

Our ELISA demonstrated good recovery rate (near 100%) using human saliva, serum, and urine-spiked oxytocin. On the other hand, the conventional method shows too low recovery rate in case of serum, and non-pretreated method shows too high recovery rate in the all samples.

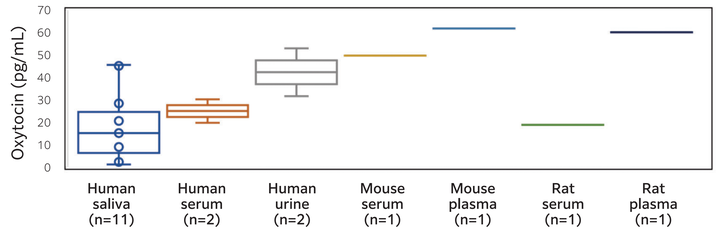


Dilution linearity

Our ELISA demonstrated good dilution linearity using human plasma, serum, urine, and saliva, and mouse/rat plasma and serum. The representative data are shown.



Example of measurement -healthy human / mouse / rat samples-



Sample concentration method

■Method for 3-fold concentration
Sample (after preparation): 360 μL
↓
Concentrated and dried by desktop centrifugal concentrator
30℃, 3 hours
↓
Dry: keep at 4℃
↓
Reconstitute the dry samples with 120 μL buffer
↓
Measured by ELISA

Sample	No.	Before Measurement (pg/mL)	After Measurement (pg/mL)	After/before
Human Saliva	1	28.4	80.5	2.83
	2	15.1	40.3	2.67
	3	20.5	55.2	2.69
Human Serum	1	48.8	122	2.50
	2	31.9	99.7	3.13
	3	33.8	95.6	2.83
Human Urine	1	36.8	105	2.85
	2	12.0	31.9	2.66
	3	44.5	151	3.39

3-fold concentration method induces about triple the measured value.
This method is useful for measuring samples with lower oxytocin concentration.

Conclusion

- **We have developed a novel oxytocin ELISA method that can measure a variety of samples using a simple pretreatment method.**
- **Our method is expected to be a useful tool for various oxytocin studies.**
- **We are developing ELISA using monoclonal antibody aiming stable supply as a next step.**

Disclosure of Conflict of Interest

Matters requiring disclosures of COI with regard to the poster are as follows:
Employee of FUJIFILM Wako Pure Chemical Corporation