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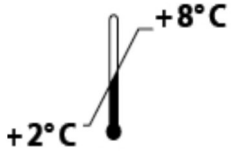
Manual

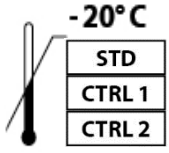
IDK® Serotonin ELISA

For the in vitro determination of serotonin (5-HT) in human serum and dried blood spots

Valid from 2020-05-08

REF **K 6880**





IVD CE

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1. INTENDED USE

This Immundiagnostik AG assay is intended for the quantitative determination of serotonin (5-hydroxytryptamine, 5-HT) in human serum and dried blood spots. For *in vitro* diagnostic use only.

2. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
K 6880	PLATE	Microtiter plate, pre-coated	12 x 8 wells
K 6880	STD	Standards, ready-to-use (0, 10, 30, 100, 250, 750 ng/ml)	6 x 200 µl
K 6880	CTRL 1	Control, ready-to-use (see specification for range)	1 x 200 µl
K 6880	CTRL 2	Control, ready-to-use (see specification for range)	1 x 200 µl
K 6880	ACTSOL	Activating solution, ready-to-use	1 x 7 ml
K 0006.C.100	WASHBUF A	Wash buffer concentrate,10 x	2 x 100 ml
K 6880	CONJ	Conjugate, peroxidase-labelled, ready-to-use	1 x 12 ml
K 6880	REABUF	Reaction buffer, ready-to-use	2 x 30 ml
K 6880	DER	Derivatisation reagent, lyophilised	1 x 100 mg
K 0008.07	DMSO	Dimethylsulfoxide (DMSO)	1 x 7 ml
K 6880	AB	Serotonin antibody, lyophilised	1 x 1 vial
K 0002.15	SUB	Substrate (tetramethylbenzidine), ready-to-use	1 x 15 ml
K 0003.15	STOP	Stop solution, ready-to-use	1 x 15 ml

For reorders of single components, use the catalogue number followed by the label as product number.

3. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultrapure water*
- Dried blood spot carrier such as DrySpot-ID cat. no. DZ9021ID prepared for serotonin

- Calibrated precision pipets and 10-1000 µl single-use tips
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- Multi-channel pipets or repeater pipets
- Vortex
- Standard single-use laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader (required filters see chapter 6)

* Immundiagnostik AG recommends the use of ultrapure water (water type 1; ISO 3696), which is free of undissolved and colloidal ions and organic molecules (free of particles > 0.2 µm) with an electrical conductivity of 0.055 µS/cm at 25 °C (≥18.2 MΩ cm).

4. STORAGE AND PREPARATION OF REAGENTS

- To run the assay more than once, ensure that reagents are stored at the conditions stated on the label.
- **Preparation of the wash buffer**: The **wash buffer concentrate (WASHBUF A)** has to be diluted with ultrapure water **1:10** before use (100 ml WASHBUF A + 900 ml ultrapure water), mix well. Crystals could occur due to high salt concentration in the concentrate. Before dilution, the crystals have to be redissolved at room temperature or in a water bath at 37 °C. The **WASHBUF A** is stable at **2-8 °C** until the expiry date stated on the label. **Wash buffer** (1:10 diluted WASHBUF A) can be stored in a closed flask at **2-8 °C for 1 month**.
- Store **standards and controls (STD/CTRL)** frozen at **-20 °C**. They are stable at -20 °C until the expiry date stated on the label. Thaw before use in the test and mix well. Re-freeze standards and controls after use.
- **DMSO** crystallises at 2-8 °C. Before use, bring to room temperature to dissolve the crystals.
- The **lyophilised derivatisation reagent (DER)** is stable at **2-8 °C** until the expiry date stated on the label. Bring to room temperature before opening and reconstitute the DER (100 mg) with **6 ml DMSO**. Allow to dissolve for 10 minutes and mix thoroughly with a vortex-mixer. **The derivatisation reagent** (reconstituted DER) **can be stored at 2-8 °C for 2 months**. Bring to room temperature before reuse. Please note: DMSO attacks all plastics but not polypropylene products and laboratory glass.

- The **lyophilised serotonin antibody (AB)** is stable at **2-8 °C** until the expiry date stated on the label. Reconstitute the AB with **6 ml of wash buffer**. **Serotonin antibody** (reconstituted AB) **can be stored at 2-8 °C for 2 months.**
- All other test reagents are ready-to-use. Test reagents are stable until the expiry date (see label) when stored at **2-8 °C.**

5. STORAGE AND PREPARATION OF SAMPLES

Serum samples

Serum samples are stable for up to 48 hours at 2-8 °C. For longer storage, store samples frozen at -20 °C. As serotonin is sensitive to light, store samples in a cool and dark place after collection.

The serum samples are analysed **undiluted**.

For sample preparation, a derivatisation reagent for derivatisation of serotonin is added (see derivatisation procedure).

Dried blood spots

Collection and storage of dried blood spots

50 µl whole blood dripped on a dried sample carrier cleared by Immundiagnostik AG are suitable as sample material after complete drying. We recommend DrySpot-ID (catalogue no. DZ9021ID prepared for serotonin) as dried blood spot carrier. The moistened cards are stable for 1 week at room temperature protected from light. For longer storage, store at -20°C in a dry place.

Preparation of the dried blood samples

1.	Remove Filter from sampling device and put it in a labelled 1.5 ml poly-propylene tube.
2.	Add **50 µl activating solution** (ACTSOL) on the dried blood sample.
3.	Allow sample to stand for **15-30 min** at room temperature (15-30 °C).

For sample preparation, a derivatisation reagent for derivatisation of serotonin is added (see derivatisation procedure).

6. ASSAY PROCEDURE

Principle of the test

This ELISA is designed for the quantitative determination of serotonin in human serum and dried blood spots. The assay is based on the method of competitive enzyme linked immunoassays.

The sample preparation includes the addition of a derivatisation reagent for serotonin derivatisation. Afterwards, the treated samples and the polyclonal serotonin antiserum are incubated in the wells of a microtiter plate coated with serotonin derivative (tracer). During the incubation period, the target serotonin in the sample competes with the tracer, immobilised on the wall of the microtiter wells, for the binding of the polyclonal antibodies.

During the second incubation step, a peroxidase-conjugated antibody is added to detect the anti-serotonin antibodies. After washing away the unbound components, tetramethylbenzidine (TMB) is added as a peroxidase substrate. Finally, the enzymatic reaction is terminated by an acidic stop solution. The colour changes from blue to yellow, and the absorbance is measured in the photometer at 450 nm. The intensity of the yellow colour is inverse proportional to the serotonin concentration in the sample; this means, high serotonin concentration in the sample reduces the concentration of tracer-bound antibodies and lowers the photometric signal. A dose response curve of the absorbance unit (optical density, OD at 450 nm) vs. concentration is generated, using the values obtained from the standard. Serotonin, present in the patient samples, is determined directly from this curve.

Derivatisation procedure

Bring **all reagents and samples to room temperature** (15-30 °C) and mix well.

Derivatisation of standards, controls and samples is carried out in single analysis in vials (e.g. 1.5 ml polypropylene vials).

We recommend preparing one derivatisation per standard, control and sample and transferring it in duplicate determinations into the wells of the microtiter plate.

1.	Add **25 µl standard** (STD)/**control** (CTRL)/**serum sample** in the corresponding vials.
2.	Add **500 µl reaction buffer** (REABUF) into each vial (STD, CTRL, serum) as well as into the vials with the prepared dried blood samples.

3.	Add **50 µl derivatisation reagent** into each vial (STD, CTRL, serum, dried blood spot) and **mix thoroughly** for several seconds on a vortex mixer or by inversion. Make sure the dry blood carriers are completely covered with liquid.
4.	Incubate for **30 min at room temperature** (15-30 °C) on a **horizontal shaker**.

2 x 50 µl of the derivatised standards, controls and samples are used in the ELISA as duplicates.

Test procedure

Mark the positions of standards/controls/samples in duplicate on a protocol sheet.

Take as many microtiter strips as needed from the kit. Store unused strips covered with foil at 2-8 °C. Strips are stable until expiry date stated on the label.

5.	For the analysis in duplicate take **2 x 50 µl** of the **derivatised standards/ controls/samples** out of the vials and add into the respective wells of the microtiter plate.
6.	Add **50 µl serotonin antibody** into each well of the microtiter plate.
7.	Cover the plate tightly with foil and incubate for **1 hour at** room temperature (15-30 °C) on a **horizontal shaker**.
8.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
9.	Add **100 µl conjugate** (CONJ) into each well.
10.	Cover the strips and incubate for **30 minutes** at room temperature (15-30 °C) on a **horizontal shaker**.
11.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
12.	Add **100 µl substrate** (SUB) into each well.
13.	Incubate for **10-14 min*** at room temperature (15-30 °C) in the **dark**.

14.	Add **100 µl stop solution** (STOP) into each well and mix well.
15.	Determine **absorption immediately** with an ELISA reader at **450 nm** against 620 nm (or 690 nm) as a reference. If no reference wavelength is available, read only at 450 nm. If the extinction of the highest standard exceeds the range of the photometer, absorption must be measured immediately at **405 nm** against 620 nm (690 nm) as a reference.

* The intensity of the colour change is temperature sensitive. We recommend observing the colour change and stopping the reaction upon good differentiation.

For automated ELISA processors, the given protocol may need to be adjusted according to the specific features of the respective automated platform. For further details please contact your supplier or Immundiagnostik AG.

7. RESULTS

The following algorithms can be used alternatively to calculate the results. We recommend using the 4 parameter algorithm.

1. **4 parameter algorithm**

   It is recommended to use a linear ordinate for optical density and a logarithmic abscissa for concentration. When using a logarithmic abscissa, the zero standard must be specified with a value less than 1 (e.g. 0.001).

2. **Point-to-point calculation**

   We recommend a linear ordinate for optical density and a linear abscissa for concentration.

3. **Spline algorithm**

   We recommend a linear ordinate for optical density and a linear abscissa for concentration.

The plausibility of the duplicate values should be examined before the automatic evaluation of the results. If this option is not available with the program used, the duplicate values should be evaluated manually.

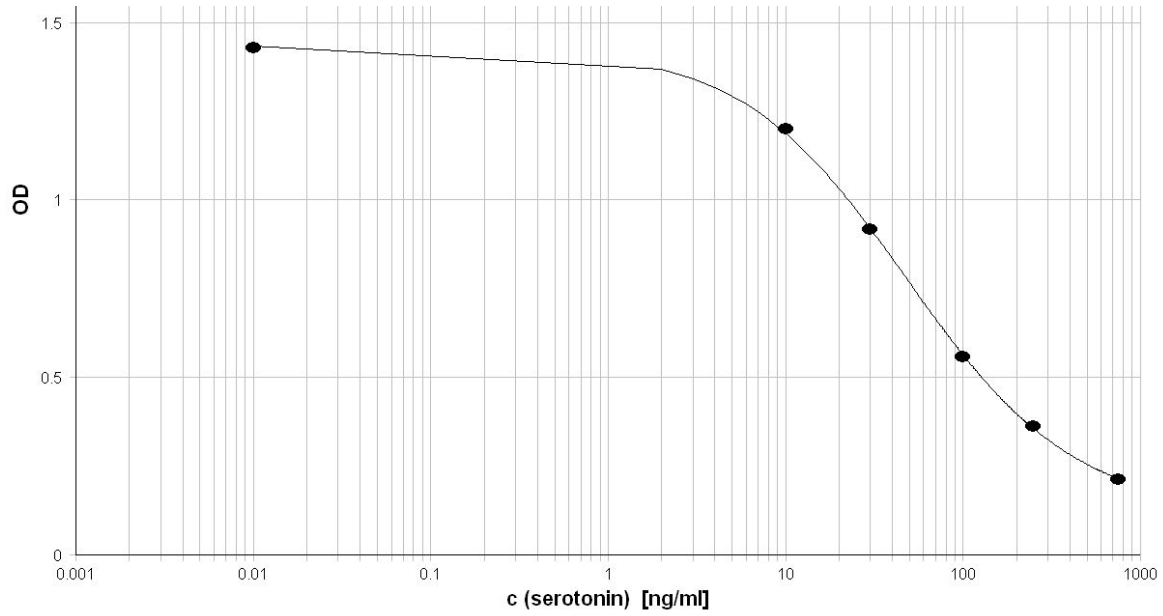
Serum

No factor is required.

Dried blood spots

The obtained results have to be multiplied by the **factor of 2.3** to get the actual concentrations.

In the following, an example of a standard curve is given. Do not use it for the calculation of your results.



8. LIMITATIONS

Samples with concentrations above the measurement range can be diluted with reaction buffer and re-assayed. Please consider this dilution factor when calculating the results.

Samples with concentrations lower than the measurement range cannot be clearly quantified.

The upper limit of the measurement range can be calculated as:

highest concentration of the standard curve × *sample dilution factor to be used*

The lower limit of the measurement range can be calculated as:

analytical sensitivity × *sample dilution factor to be used*

Analytical sensitivity see chapter "Performance Characteristics".

Biotin interference

Samples containing a biotin concentration of < 400 ng/ml show a change of the results of ≤ 25 %. Higher concentrations of biotin can lead to falsely low results. Patients taking > 5 mg biotin per day should wait at least 24 hours after taking biotin to have their samples collected. Results of patients taking biotin supplements or receiving a high-dose biotin therapy should generally be interpreted along with the total clinical picture.

9. QUALITY CONTROL

Immundiagnostik AG recommends the use of external controls for internal quality control, if possible.

Control samples should be analysed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the patient samples may not be valid if within the same assay one or more values of the quality control samples are outside of the acceptable limits.

Reference Range

Based on internal studies with samples from apparently healthy persons (n = 40), the following normal range was estimated:

Mean value:	160 ng/ml
10^{th} percentile:	88 ng/ml
90^{th} Percentile:	232 ng/ml

We recommend each laboratory to establish its own reference range.

10. PERFORMANCE CHARACTERISTICS

Accuracy – Precision

Repeatability (Intra-Assay); n = 12

The repeatability was assessed with 2 control samples under constant parameters (same operator, measurement system, day and kit lot).

sample	mean value [ng/ml]	CV [%]
1	48.1	5.2
2	107.1	8.1

Reproducibility (Inter-Assay); n = 15

The reproducibility was assessed with 3 serum samples under varying parameters (different operators, measurement systems, days and kit lots).

sample	mean value [ng/ml]	CV [%]
1	92.4	9.9
2	65.8	10.6
3	59.4	10.9

Accuracy – Trueness

The trueness states the closeness of the agreement between the result of a measurement and the true value of the measurand. Therefore, serotonin spikes with known concentrations were added to 2 different serum samples. The following values have been obtained:

sample [ng/ml]	spike [ng/ml]	expected [ng/ml]	obtained [ng/ml]	recovery [%]
98.7	25	123.7	129.4	104.6
	50	148.7	153.9	103.5
	100	198.7	180.8	91.0
72.8	25	97.8	102.3	104.6
	50	122.8	120.4	98.1
	100	172.8	168.1	97.3

Linearity

The linearity states the ability of a method to provide results proportional to the concentration of analyte in the test sample within a given range. This was assessed with a serial dilution of 4 serum samples.

For Serotonin in serum, the method has been demonstrated to be linear from 17.7 to 473.2 ng/ml, showing a non-linear behaviour of less than ±20 % in this interval.

sample [ng/ml]	dilution	expected [ng/ml]	obtained [ng/ml]	recovery [%]
70.6	1:2	35.3	36.0	102.1
	1:3	23.5	22.5	95.6
	1:4	17.7	16.3	92.4
109.1	1:2	54.6	58.6	107.4
	1:3	36.4	38.4	105.5
	1:4	27.3	24.5	89.9
368.8	1:2	184.4	167.4	90.8
	1:3	122.9	112.6	91.6
	1:4	92.2	86.6	94.0
473.2	1:2	236.6	226.1	95.6
	1:3	157.7	143.7	91.1
	1:4	118.3	111.3	94.1

Analytical sensitivity

The zero-standard was measured 81 times. The detection limit was set as B_0 - 2 SD and estimated to be 6.9 ng/ml.

Analytical specificity

The specificity of the antibody was tested by measuring the cross-reactivity against a range of compounds with structural similarity to serotonin. The specificity is calculated in percent in relation to the serotonin-binding activity:

3-Indoleacrylic acid	< 0.04%
Indole-3-pyruvic acid	< 0.03%
3-Indoleacetic acid	< 0.05 %
5-Methoxytryptophol	< 0.15 %
L-5-OH-tryptophan	< 0.21 %

11. PRECAUTIONS

- All reagents in the kit package are for *in vitro* diagnostic use only.

- Kit reagents contain sodium azide or ProClin as bactericides. Sodium azide and ProClin are toxic. Substrates for the enzymatic colour reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.
- The stop solution consists of diluted sulfuric acid, a strong acid. Although diluted, it still must be handled with care. It can cause burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spill should be wiped up immediately with copious quantities of water. Do not breathe vapour and avoid inhalation.

12. TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay. Furthermore, we recommend not assembling wells of different microtiter plates for analysis, even if they are of the same batch.
- Control samples should be analysed with each run.
- Reagents should not be used beyond the expiration date stated on the kit label.
- Substrate solution should remain colourless until use.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.
- Do not mix plugs and caps from different reagents.
- The assay should always be performed according to the enclosed manual.

13. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and distributed according to the IVD guidelines of 98/79/EC.
- The guidelines for medical laboratories should be followed.
- *IDK*® is a trade mark of Immundiagnostik AG.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from incorrect use.

- Warranty claims and complaints regarding deficiencies must be logged within 14 days after receipt of the product. The product should be sent to Immundiagnostik AG along with a written complaint.

14. REFERENCES

1. Badawy, A. a-B. Tryptophan: the key to boosting brain serotonin synthesis in depressive illness. *Journal of psychopharmacology (Oxford, England)* **27,** 878–93 (2013).
2. Gershon, M. D. 5-Hydroxytryptamine (serotonin) in the gastrointestinal tract. *Curr Opin Endocrinol Diabetes Obes* **20,** 14–21 (2013).
3. Gershon, M. D. Serotonin is a sword and a shield of the bowel: serotonin plays offense and defense. *Transactions of the American Clinical and Climatological Association* **123,** 268–80; discussion 280 (2012).
4. Namkung, J., Kim, H. & Park, S. Peripheral Serotonin: a New Player in Systemic Energy Homeostasis. *Molecules and Cells* **38,** 1–6 (2015).
5. Park, H.-J., Lee, S.-E., Oh, J.-H., Seo, K.-W. & Song, K.-H. Leptin, adiponectin and serotonin levels in lean and obese dogs. *BMC veterinary research* **10,** 113 (2014).
6. Reigstad, C. S., Salmonson, C. E., Rainey, J. F., Szurszewski, J. H., Linden, D. R., Sonnenburg, J. L., Farrugia, G. & Kashyap, P. C. Gut microbes promote colonic serotonin production through an effect of short-chain fatty acids on enterochromaffin cells. *The FASEB Journal* **29,** 1395–1403 (2015).

Used symbols:

 Temperature limitation

Catalogue Number

In Vitro Diagnostic Medical Device

To be used with

 Manufacturer

Contains sufficient for <n> tests

Lot number

 Use by

 Attention

 Consult instructions for use

Consult specification data sheet