



Distribuito in ITALIA da  
**Li StarFish S.r.l.**  
Via Cavour, 35  
20063 Cernusco S/N (MI)  
telefono 02-92150794  
info@listarfish.it  
www.listarfish.it

**Manual**

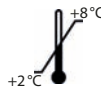
# CRP ELISA

*For the in vitro determination of C-reactive protein in  
serum, plasma, stool, dried blood spots and urine*

Valid from 2019-07-18



**K 9710s**



**Immundiagnostik AG**, Stubenwald-Allee 8a, 64625 Bensheim, Germany

Tel.: +49 6251 70190-0

Fax: + 49 6251 70190-363

e.mail: info@immundiagnostik.com

www.immundiagnostik.com

# Table of Contents

<b>1. INTENDED USE</b>	<b>19</b>
<b>2. INTRODUCTION</b>	<b>19</b>
<b>3. MATERIAL SUPPLIED</b>	<b>19</b>
<b>4. MATERIAL REQUIRED BUT NOT SUPPLIED</b>	<b>20</b>
<b>5. STORAGE AND PREPARATION OF REAGENTS</b>	<b>20</b>
<b>6. STORAGE AND PREPARATION OF SAMPLES</b>	<b>21</b>
<i>Serum and plasma samples</i>	21
<i>Dried blood spots</i>	22
<i>Urine samples</i>	22
<i>Stool samples</i>	23
<b>7. ASSAY PROCEDURE</b>	<b>24</b>
<i>Principle of the test</i>	24
<i>Test procedure</i>	24
<b>8. RESULTS</b>	<b>26</b>
<b>9. LIMITATIONS</b>	<b>27</b>
<b>10. QUALITY CONTROL</b>	<b>27</b>
<i>Reference range</i>	27
<b>11. PERFORMANCE CHARACTERISTICS</b>	<b>28</b>
<i>Accuracy – Precision</i>	28
<i>Analytical sensitivity</i>	28
<i>Analytical specificity</i>	28
<i>Accuracy – Trueness</i>	29
<i>Linearity</i>	30
<b>12. PRECAUTIONS</b>	<b>31</b>
<b>13. TECHNICAL HINTS</b>	<b>31</b>
<b>14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE</b>	<b>32</b>
<b>15. REFERENCES</b>	<b>32</b>

## 1. INTENDED USE

This Immundiagnostik AG assay is a enzyme immunoassay intended for the quantitative determination of C-reactive protein in plasma, serum, stool, dried blood spots, and urine. It is for *in-vitro* diagnostic use only.

## 2. INTRODUCTION

C-reactive protein (CRP) is mainly formed in hepatocytes. The synthesis rate of CRP is influenced by the cytokines involved in the inflammatory processes. The biological half-life time is estimated to be 13–16 hours. The serum CRP concentration reflects very sensitive acute fever, pneumonia and myocardial infarction.

Studies describe an association between inflammatory reactions and cardiovascular diseases like arteriosclerosis or latent and chronic persistent infections. As a marker for inflammation, CRP high-sensitive can be used to predict the risk of myocardial infarction and stroke.

The CRP determination in urine using the high sensitive ELISA method allows – together with  $\alpha_2$ -macroglobulin – an early screening diagnose after kidney transplantations. The CRP kit provides an easy to use assay for monitoring anti-rejection therapies. ELISA assay was used for years to test hundreds of patients. Its predictive diagnostic value was compared with the gold standard (kidney biopsy).

### Indications

- Prognosis factor for myocardial infarction or stroke
- Inflammatory processes

## 3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
K 9710s	PLATE	Microtiter plate, pre-coated	12 x 8 wells
K 0001.C.100	WASHBUF	Wash buffer concentrate, 10x	1 x 100 ml
K 9710s	CONJ	Conjugate, (rabbit-anti-CRP-antibody, peroxidase-labelled)	1 x 150 $\mu$ l
K 9710s	STD	Standards*, ready-to-use, (0; 1.9; 5.6; 16.7; 50; 150 ng/ml)	6 x 1 ml
K 9710s	CTRL 1	Control, ready-to-use (see specification for range)	1 x 1 ml
K 9710s	CTRL 2	Control, ready-to-use (see specification for range)	1 x 1 ml

Cat. No.	Label	Kit components	Quantity
K 9710s	SAMPLEBUF	Sample dilution buffer, ready-to-use	2 x 100 ml
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

\*The CRP calibrators were standardized against WHO standard 470.

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

#### 4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultrapure water\*
- Dried blood spot carrier such as DrySpot-ID cat. no.: DZ9020ID or DZ9021ID
- Stool sample application system such as Cat. No.: K 6998SAS
- Calibrated precision pipettors and 10–1000 µl single-use tips
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- Multi-channel pipets or repeater pipets
- Centrifuge
- Vortex
- Standard single-use laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader (required filters see chapter 7)

\* 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).

#### 5. STORAGE AND PREPARATION OF REAGENTS

- To run the assay more than once, ensure that reagents are stored at the conditions stated on the label. **Prepare only the appropriate amount necessary for each run.** The kit can be used up to 4 times within the expiry date stated on the label.
- Reagents with a volume less than **100 µl** should be centrifuged before use to avoid loss of volume.
- **Preparation of the wash buffer:** The **wash buffer concentrate (WASHBUF)** has to be diluted with ultrapure water **1:10** before use (100 ml WASHBUF + 900 ml ultrapure water), mix well. Crystals could occur due to high salt concentration in the concentrate. Before dilution, the crystals have to be redis-

solved at room temperature or in a water bath at 37°C. The **WASHBUF** is stable at **2–8°C** until the expiry date stated on the label. **Wash buffer** (1:10 diluted WASHBUF) can be stored in a closed flask at **2–8°C for 1 month**.

- **Preparation of the conjugate:** Before use, the **conjugate concentrate (CONJ)** has to be diluted **1:101** in **wash buffer** (100 µl CONJ + 10 ml wash buffer). The CONJ is stable at **2–8°C** until the expiry date stated on the label. **Conjugate (1:101 diluted CONJ) is not stable and cannot be stored.**
- All other test reagents are ready-to-use. Test reagents are stable until the expiry date (see label) when stored at **2–8°C**.

## 6. STORAGE AND PREPARATION OF SAMPLES

### *Serum and plasma samples*

#### **Collection and storage of samples**

##### **Collection of serum**

Collect sufficient blood (at least 1 ml) by venipuncture into a tube or a plastic syringe, avoid hemolysis, allow to stand for 15 min, centrifuge for 15 min at 1 000 g and 4°C and collect the serum.

##### **Collection of plasma**

Collect sufficient blood (at least 1 ml) by venipuncture into an EDTA venipuncture tube or a plastic syringe, allow to stand for 15 min, centrifuge for 15 min at 1 000 x g and 4°C and separate the plasma from the cells.

##### **Storage of serum**

Serum samples can be stored at -80°C for 11 years.\*

\*A. P. Doumatey et al. 2014.

##### **Sample dilution**

Serum and plasma samples have to be diluted **1:100 or 1:500** before performing the assay.

For a dilution of 1:100 e.g.:

Add **10 µl serum /plasma to 990 µl sample dilution buffer (SAMPLEBUF)** and mix well.

Use the dilution factor (100 or 500) to calculate the CRP concentration read off the calibration curve.

Patient's samples with **elevated CRP concentrations** must be diluted **1:4 000–1:8 000**. Samples of other patient collectives must be diluted according to the ex-

pected CRP concentration. The corresponding dilution factor must be used for calculation of the CRP concentration.

### *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 DZ9020ID or DZ9021ID) as dried blood spot carrier. The moistened cards are stable for 3 weeks at room temperature.

#### **Preparation of the dried blood samples**

1.	Label 1,5 ml polypropylene tubes.
2.	Remove filter from sampling device.
3.	Put filter in a labelled tube.
4.	Add <b>1000 µl</b> sample dilution buffer (SAMPLEBUF) to each sample, allow sample to stand for <b>15 min</b> at room temperature (15–30 °C).
5.	Vortex for <b>10 s</b> . The filter will decolourise.
6.	Centrifuge the samples for <b>5 min</b> at <b>3000 g</b> to remove residual filter pieces.

For testing in duplicates, pipet 2 x 100 µl of each prepared sample per well.

### *Urine samples*

#### **Storage of urine samples**

Urine should be stored at –20 °C until the measurement. CRP in urine is stable for 4 weeks at –20 °C.

#### **Dilution of urine samples**

Urine samples must be diluted **1:5** before performing the assay,

e.g. **50 µl** sample + **200 µl** sample dilution buffer (SAMPLEBUF), mix well.

For analysis, pipet 100 µl of this dilution per well.

## *Stool samples*

### **Storage of stool samples**

The samples should be refrigerated and can be stored at **2–8 °C for 2 days**. If the test cannot be performed within this period, the specimen should be stored at –20 °C or colder. At this temperature, they are stable for up to 2 months.

### **Extraction of the stool samples**

**Wash buffer** (1:10 diluted WASHBUF) is used as a **sample extraction buffer**. We recommend the following sample preparation:

### **Stool Sample Application System (SAS) (Cat. No.: K 6998SAS)**

#### ***Stool sample tube – Instructions for use***

Please note that the dilution factor of the final stool suspension depends on the amount of stool sample used and the volume of the buffer.

#### ***SAS with 0,75 ml wash buffer:***

Applied amount of stool:	15 mg
Buffer Volume:	0,75 ml
Dilution Factor:	1:50

Please follow the instructions for the preparation of stool samples using the SAS as follows:

- The raw stool sample has to be thawed. For particularly heterogeneous samples we recommend a mechanical homogenisation using an applicator, inoculation loop or similar device.
- Fill the **empty stool sample tube** with 0.75 ml **wash buffer** (1:10 diluted WASHBUF) before using it with the sample. **Important:** Allow the wash buffer to reach room temperature.
- Unscrew the tube (yellow part of cap) to open. Insert the yellow dipstick into the sample. The lower part of the dipstick has notches which need to be covered completely with stool after inserting it into the sample. Place dipstick back into the tube. When putting the stick back into the tube, excess material will be stripped off, leaving 15 mg of sample to be diluted. Screw tightly to close the tube.
- Shake the tube well until no stool sample remains in the notches. **Important:** Please make sure that you have a maximally homogenous suspension after shaking. Especially with more solid samples, soaking the sample in the tube with sample extraction buffer for ~ 10 minutes improves the result.

- e) Allow sample to stand for ~10 minutes until sediment has settled. Floating material like shells of grains can be neglected.
- f) Carefully unscrew the complete cap of the tube including the blue ring plus the dipstick. Discard cap and dipstick. Make sure that the sediment will not be dispersed again.

**Dilution:** 1:50

The extract can be stored **1 month at -20°C**.

**100 µl** per well of this supernatant is used in the assay.

## 7. ASSAY PROCEDURE

### *Principle of the test*

This ELISA is a sandwich assay for the determination of CRP in serum, plasma, dried blood spots, urine, and stool samples. The wells of the microtiter plate are coated with antibodies directed against C-reactive protein. In a first incubation step, the CRP in the samples is bound to the coated capturing antibodies (in excess). To remove all unbound substances, a washing step is carried out.

In a second incubation step, a peroxidase-labelled detection antibody is added. After another washing step, to remove all unbound substances, the solid phase is incubated with the substrate, tetramethylbenzidine. An acidic stopping solution is then added. The color converts to yellow. The intensity of the yellow color is directly proportional to the concentration of CRP in the sample. A dose response curve of the absorbance (at 450 nm) unit vs. concentration is generated. CRP, present in the patient samples, is determined directly from this calibration curve.

The combination of two specific antibodies in the CRP ELISA drastically reduces the possibility of wrong-negatives results and offers a secure diagnostic system to the user.

### *Test procedure*

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

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

Take as many microtiter strips as needed from the kit. Store unused strips together with the desiccant bag in the closed aluminium packaging at 2–8°C. Strips are stable until expiry date stated on the label.



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.

We recommend to carry out the tests in duplicate.

1.	<b>Before use</b> , wash the wells <b>5 times</b> with <b>250 µl wash buffer</b> . After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
2.	Add each <b>100 µl standards/controls/prepared samples</b> into the respective wells.
3.	Cover the strips and incubate for <b>1 h</b> at room temperature (15–30 °C) on a <b>horizontal shaker*</b> .
4.	Discard the content of each well and wash <b>5 times</b> with <b>250 µl wash buffer</b> . After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
5.	Add <b>100 µl conjugate</b> (diluted CONJ) into each well.
6.	Cover the strips and incubate for <b>1 h</b> at room temperature (15–30 °C) on a <b>horizontal shaker*</b> .
7.	Discard the content of each well and wash <b>5 times</b> with <b>250 µl wash buffer</b> . After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
8.	Add <b>100 µl substrate</b> (SUB) into each well.
9.	Incubate for <b>10–20 min**</b> at room temperature (15–30 °C) in the <b>dark</b> .
10.	Add <b>100 µl stop solution</b> (STOP) into each well and mix well.
11.	Determine <b>absorption immediately</b> with an ELISA reader at <b>450 nm</b> 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 <b>405 nm</b> against 620 nm as a reference.

\* We recommend shaking the strips at 550 rpm with an orbit of 2 mm.

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

## 8. 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 the optical density and a logarithmic abscissa for the 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 the optical density and a linear abscissa for the concentration.

### 3. Spline algorithm

We recommend a linear ordinate for the optical density and a linear abscissa for the 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 programme used, the duplicate values should be evaluated manually.

### Serum and plasma

The obtained results have to be multiplied by the **dilution factor of 100 or 500** to get the actual concentrations.

If samples were diluted **1:4 000** or **1:8 000**, the obtained results have to be multiplied by the **dilution factor of 4 000 or 8 000** respectively.

### Dried blood spots

The obtained results have to be multiplied by the **dilution factor of 60** to get the actual concentrations.

### Urine

The obtained results have to be multiplied by the **dilution factor of 5** to get the actual concentrations.

### Stool

The obtained results have to be multiplied by the **dilution factor of 50** to get the actual concentrations.

In case **another dilution factor** has been used, multiply the obtained result with the dilution factor used.

## 9. LIMITATIONS

Samples with concentrations above the measurement range (see definition below) can be further diluted and re-assayed. Please consider this higher dilution when calculating the results.

Samples with concentrations lower than the measurement range (see definition below) 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".

## 10. 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 sample are outside the acceptable limits.

### *Reference range*

#### **CRP concentration serum/plasma\*/dried blood spots**

- < 1 mg/l      low CHD-Risk
- 1–3 mg/l     medium CHD-Risk
- > 3 mg/l     high CHD-Risk

\*Pearson et al., 2003

#### **CRP concentration stool**

< 56 ng/ml

#### **CRP concentration urine**

< 6 ng/ml

If the serum/plasma CRP concentration is found to be **higher than 3 mg/l**, a second determination should be made within 2 to 3 weeks. If the CRP concentration is high again, and other reasons can be excluded (acute infection, chronic-inflammatory dis-

eases), the obtained CRP concentration can be used for risk stratification in coronary heart disease (CHD) patients. If the CHD risk is high, the lifestyle should be changed together with medical treatment. These normal ranges should be used as a guideline only.

We recommend each laboratory to establish its own reference range.

## 11. PERFORMANCE CHARACTERISTICS

### *Accuracy – Precision*

#### **Repeatability (Intra-Assay); n = 42**

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

Sample	Mean value [ng/ml]	CV [%]
1	232.5	6.7
2	994.5	6.8

#### **Reproducibility (Inter-Assay); n = 25**

The reproducibility was assessed with 2 serum samples under varying parameters (different operators, measurement systems, days and kit lots).

Sample	Mean value [ng/ml]	CV [%]
1	4.98	9.9
2	35.84	7.3

### *Analytical sensitivity*

The zero-standard was measured 42 times. The detection limit was set as  $B_0 + 2 \text{ SD}$  and estimated to be 1.015 ng/ml without considering possibly used sample dilution factors.

### *Analytical specificity*

The specificity of the antibody was tested by measuring the cross-reactivity against substances with structural similarity to CRP. There was no cross-reactivity observed with alpha-1-antitrypsin, lysozyme or albumin. Additionally, no cross reactivity with CRP in mouse serum was observed.

### Accuracy – Trueness

The trueness states the closeness of the agreement between the result of a measurement and the true value of the measurand. Therefore, CRP spikes with known concentrations were added to 3 different serum samples. The following values have been obtained without consideration of possibly used dilution factors:

Sample [ng/ml]	Spike [ng/ml]	Expected [ng/ml]	Obtained [ng/ml]	Recovery [%]
46.75	150.0	196.75	178.32	90.63
	75.0	121.75	136.42	112.05
	37.5	84.25	97.07	115.21
	18.8	65.50	72.92	111.31
	9.4	56.13	63.36	112.88
	4.7	51.44	60.98	118.53
	2.3	49.10	57.01	116.12
10.92	150.0	160.92	151.80	94.33
	75.0	85.92	95.20	110.80
	37.5	48.42	52.41	108.24
	18.8	29.67	30.42	102.52
	9.4	20.29	21.61	106.51
	4.7	15.60	15.45	99.03
	2.3	13.26	13.29	100.21
8.79	150.0	156.70	158.79	98.68
	75.0	96.20	83.79	114.80
	37.5	44.52	46.29	96.16
	18.8	27.34	27.54	99.25
	9.4	18.13	18.17	99.77
	4.7	14.28	13.48	105.89
	2.3	12.10	11.14	108.63

### 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 according to CLSI guideline EP6-A with a serial dilution of each one serum, plasma and stool sample.

For CRP in serum, plasma, urine and stool, the method has been demonstrated to be linear from 1.15 to 178.75 ng/ml based on the standard curve without considering possibly used sample dilution factors, showing a non-linear behaviour of less than  $\pm 20\%$  in this interval.

Sample	Dilution	Expected [ng/ml]	Obtained [ng/ml]	Recovery [%]
Serum	1:800	178.75	178.75	-
	1:1600	89.38	95.63	106.99
	1:3200	44.69	40.63	90.91
	1:6400	22.34	18.59	83.22
	1:12800	11.17	8.91	79.72
Plasma	1:100	76.59	76.59	-
	1:200	38.30	41.70	108.90
	1:400	19.15	19.14	99.97
	1:500	15.32	17.16	112.01
	1:1000	7.66	7.51	98.07
	1:2000	3.83	3.29	85.99
Stuhl	1:100	36.64	36.64	-
	1:200	18.32	19.04	103.95
	1:400	9.16	8.97	97.89
	1:800	4.58	4.41	96.19
	1:1600	2.29	2.29	99.78
	1:3200	1.15	0.99	86.15

## 12. PRECAUTIONS

- All reagents in the kit package are for *in vitro* diagnostic use only.
- Human materials used in kit components were tested and found to be negative for HIV, Hepatitis B and Hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious.
- 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 sulphuric acid, a strong acid. Although diluted, it still should 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 breath vapour and avoid inhalation.

## 13. 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.

## 14. 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.
- 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.

## 15. REFERENCES

1. Doumatey, Ayo P, Jie Zhou, Adebowale Adeyemo, and Charles Rotimi. 2014. "High Sensitivity C-Reactive Protein (Hs-CRP) Remains Highly Stable in Long-Term Archived Human Serum." *Clinical Biochemistry* **47** (4-5) (March): 315–8. doi:10.1016/j.clinbiochem.2013.12.014.
2. Koenig, Wolfgang, Hannelore Löwel, Jens Baumert, and Christa Meisinger. 2004. "C-Reactive Protein Modulates Risk Prediction Based on the Framingham Score: Implications for Future Risk Assessment: Results from a Large Cohort Study in Southern Germany." *Circulation* **109** (11) (March 23): 1349–53. doi:10.1161/01.CIR.0000120707.98922.E3.
3. Pearson, T. a. 2003. "American Heart Association Guide for Improving Cardiovascular Health at the Community Level: A Statement for Public Health Practitioners, Healthcare Providers, and Health Policy Makers From the American Heart Association Expert Panel on Population and Pre." *Circulation* **107** (4) (February 4): 645–651. doi:10.1161/01.CIR.0000054482.38437.13.
4. Ridker, P M, C H Hennekens, J E Buring, and N Rifai. 2000. "C-Reactive Protein and Other Markers of Inflammation in the Prediction of Cardiovascular Disease in Women." *The New England Journal of Medicine* **342** (12) (March 23): 836–43. doi:10.1056/NEJM200003233421202.
5. Salzer, Jonatan, Göran Hallmans, Maria Nyström, Hans Stenlund, Göran Wadell, and Peter Sundström. 2013. "Vitamin A and Systemic Inflammation as Protective Factors in Multiple Sclerosis." *Multiple Sclerosis (Houndmills, Basingstoke, England)* **19** (8) (July 18): 1046–51. doi:10.1177/1352458512472752.



**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		