



ChronoCheck

**Saliva Collection & Transportation Kit
(for subsequent Melatonin assessment)**

B-CC3-U
3 Collection Sets

Version: 02
Revision date: 2026-07-04

ENGLISH

INTENDED USE

The ChronoCheck Saliva Collection Kit is intended for the accurate collection and handling of human saliva samples for the subsequent quantitative determination of the dim-light melatonin onset (DLMO) in human beings (1-6).

Subsequent quantification of melatonin in saliva can be done with one of the NovoLytiX Melatonin ELISAs or the Direct Saliva Melatonin RIA. The quantification and the required tools therefore are not part of this sampling kit. All basic information concerning the subsequent quantification steps can be found in the corresponding instructions for use of the melatonin immunoassays.

The ChronoCheck concept is schematically shown in Appendix II (page 6).

DLMO can be calculated with the help of this manual.

BACKGROUND INFORMATION

DLMO is an important parameter for the diagnosis and treatment of circadian rhythm disorders. Endogenous melatonin, a hormone produced by the pineal gland during the dark phase of the day-night cycle, synchronizes circadian rhythms, especially the sleep/wake rhythm. Its secretion is controlled by an endogenous oscillator that is entrained by light. Melatonin concentration reaches the maximum saliva levels around 03:00 to 04:00 am, varying with chronotype, whereas diurnal levels are low to undetectable.

When endogenous melatonin starts to rise (too) late, sleep/wake rhythm is delayed. *Vice versa* when it starts to raise (too) early, sleep wake rhythm is advanced. The melatonin rhythm correlates strongly with circadian rhythms. Therefore, DLMO is the most useful parameter to establish circadian rhythm disorders (4).

The determination of Melatonin concentration in Saliva is a non-invasive method. With the ChronoCheck Saliva Collection Kit it is possible to determine a partial 6-points Melatonin profile around the subject's sleep time (and if required two more samples the morning after the sampling night to estimate the Melatonin offset). The first sample should be collected four hours before the patient's habitual bed or sleep time, proceeded with a hourly saliva collection for another 5-times (and two more the next morning immediately after waking up). Using such a 6-points partial profile the determination of DLMO is possible in more than 90% of all patients. For this purpose, a total of 8 Saliva Collection Devices (Salivettes®) is included in this Saliva Collection Kit.

COLLECTION KIT MATERIALS SUPPLIED

Each B-CC3-U kit contains the materials for shipping and collection of up to 8 saliva samples for 3 patients (Table 1).

Components	Quantity	Comments
Safety box	3	Each box contains: 8 Salivettes®
White paper air-cushion envelope	3	For shipment of the tool kit to the patient; this envelope can also be used for the

		international shipment of the sampled Salivettes to the testing laboratory. In the latter case the envelope must be labeled with a UN3373 sticker.
Blue plastic safety envelope	3	Safety envelope to pack the safety box
Patient manual (saliva collection instructions)	3	Instructions for patients
Patient data form	3	Summary of patient data and collection date/times

Table 1

The contents of the ChronoCheck Saliva Collection & Transportation Kit are schematically shown in Appendix III (page 7).

MATERIALS REQUIRED BUT NOT PROVIDED

ChronoCheck is a concept for the determination and calculation of the individual DLMO and contains the required sample collection devices, safety boxes, envelopes for sample shipments and respective instructions.

For the subsequent quantification of melatonin in the saliva samples the use of one of the NovoLytiX immunoassays is strongly recommended:

- Direct Saliva Melatonin ELISA (order code: EK-DSM);
- Melatonin ELISA (order code: MLTN-96); or
- Direct Saliva Melatonin RIA (order code: RK-DSM2).

PROCEDURAL NOTES AND PRECAUTIONS FOR SPECIMEN COLLECTION

Collect saliva using the Saliva Collection Device (Salivette®) delivered with the ChronoCheck Kit. One of these cotton swabs can absorb up to 3 ml of saliva. The melatonin quantification procedure calls for 0.25 to 1.0 ml of saliva depending on the immunoassay used for melatonin determination.

Saliva collection is simple and can be performed in subjects' home at her/his convenience, but only following the subsequent precautions is essential to get reliable results.

Preparation: Subject should set aside an evening for this test avoiding sporting activities and any intense efforts as far as possible.

Light: Bright light can suppress melatonin production. Therefore, it is important to avoid bright light during the test session. Muted lighting from a reading lamp or from the television is preferable.

Eating: Nothing should be eaten during the collection time. The last meal must be taken at least 30 minutes before starting the collection. Bananas and chocolate should not be eaten during the entire day before the collection.

Drinking: Drinks containing artificial colorants, caffeine (coffee, black or green tea, iced tea, cola) or alcohol are not allowed on the evening of the collection.

Medicines: On the collection day, if possible, no aspirin and medicines that contain ibuprofen (Algiofor, Brufen, Dysmenol, Dolocyl, Ecoprofen) should be taken. If subject's sleep or sleep-wake rhythm is treated with **melatonin and/or a melatonin analogue**, such drugs **must be discontinued at least 48 hours before the collection**.

COLLECTION SCHEME

Six (or eight) saliva samples must be collected during one evening (and favourably the next morning). The time points of collection are depending on individual habitual bed and sleep time, respectively.

The first collection should be done at least four hours before the regular individual bed and sleep time, respectively. The last collection point should be collected one hour after the regular bed and sleep time and in the morning, respectively.

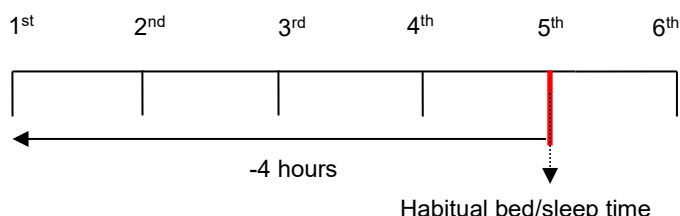
Example for a 6-point melatonin profile: If the patient's regular bed and sleep time, respectively, is 11 pm, the first collection point should be set at 7 pm, afterwards every hour one saliva sample should be collected (at 8 pm, 9 pm, 10 pm, 11 pm and 12 pm).

Note: Ideally two more samples should be taken the morning after the sampling night, the first just after waking up, the second one hour later, in order to estimate DLMO offset.

Example for Collection Times:

Bed/Sleep time	1 st	2 nd	3 rd	4 th	5 th	6 th
9 pm	5pm	6pm	7pm	8pm	9pm	10pm
10 pm	6pm	7pm	8pm	9pm	10pm	11pm
11 pm	7pm	8pm	9pm	10pm	11pm	12pm
12 pm	8pm	9pm	10pm	11pm	12pm	1am
1 am	9pm	10pm	11pm	12pm	1am	2am

Collection Points:



COLLECTION PROCEDURE



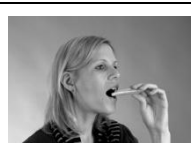
1. Before you begin the collection, label the Salivettes. Label the first Salivette with subject ID number assigned to you (or your initials), your date of birth, the day you started the saliva collection and the first planned collection time. Then label the Salivettes 2 – 6 accordingly.



2. 15 minutes before each saliva sample, you should rinse your mouth thoroughly with water.



3. Open the cap of the Salivette (the cotton swab is located in the upper part) and remove the cap from the Salivette.



4. Put the cotton swab into your mouth straight from the Salivette without touching it with your fingers.



5. Put the cotton swab between your teeth and cheek and move it around with your tongue for 2 to 3 minutes, until the cotton roll is thoroughly soaked with saliva.



6. Put the cotton swab straight into the upper part of the Salivette® from your mouth, without touching it with your fingers.



7. Using the cap, push the cotton swab into the Salivette® and close with the cap.



8. Repeat the sequences 2. to 7. once every hour to obtain the 6 saliva samples.

9. After having finished the collection period, put all tubes into the transparent safety plastic box. Put the transparent safety plastic box into the blue safety envelope.

10. Store the blue safety envelope containing the transparent plastic box with the 6 sampled Salivettes in the refrigerator for up to two days until sending it back to the Sleep Doctor or the melatonin testing laboratory.

11. Although the salivary melatonin samples are stable for several days at ambient temperatures, it is recommended to send the samples to the laboratory or to your doctor's office on Mondays to Thursdays only. Label the envelope with the corresponding address, frank the envelope (if applicable) and put it into the next mailbox; or bring it to the testing laboratory or your doctor's office.

SPECIMEN STORAGE AND SHIPMENT

Shipment: The safety box, containing the 6 to 8 labelled and sampled Salivettes must be kept in the refrigerator at approximately 2-8°C until shipment. The safety box with the Salivettes must be put into the blue safety envelope and sent by mail to the analyzing laboratory or to the doctor's office. Alternatively, the packaged Salivettes may be brought personally to the analyzing laboratory or to the doctor's office.

Attention: Samples should not be sent on Thursdays, Fridays, Saturdays or the day before holidays. The samples may be kept in the home's refrigerators for up to 3 days after collection

If the identification numbers (or your initials), birth date and collection time/date are missing and/or the labels are incorrectly filled otherwise, the laboratory may not be allowed

to carry out the melatonin determination, since there would be a risk for mixing up sample sequence and/or subject's identity.

Storage: The saliva samples absorbed to the cotton swab may be stored in the saliva collection device (Salivette®) for up to 5 days at 2-8°C. If not assayed within 5 days after collection, samples should be frozen and can be stored for up to 12 months at ≤ -20°C. Repeated freeze-thaw cycles should be avoided.

SAMPLE RECOVERY FROM SALIVETTES®

Centrifuge the Salivettes® sent by the patient for 5 min at 2000 rpm (~1000x g). Discard the suspended insert with the cotton swab, re-cap the Salivette® and store it for up to 3 days at 2-8°C or -20°C for prolonged storage up to 12 months.

SAMPLE PRETREATMENT AND ASSAY PROCEDURE

Please refer to the corresponding instruction for use of the Melatonin immunoassay of choice (see chapter “Materials Required But Not Provided”).

CALCULATION OF THE DLMO

The determination and calculation of the ELISA or RIA results is explained profoundly in the corresponding instruction for use of the method of choice (see also chapter “Materials Required But Not Provided”).

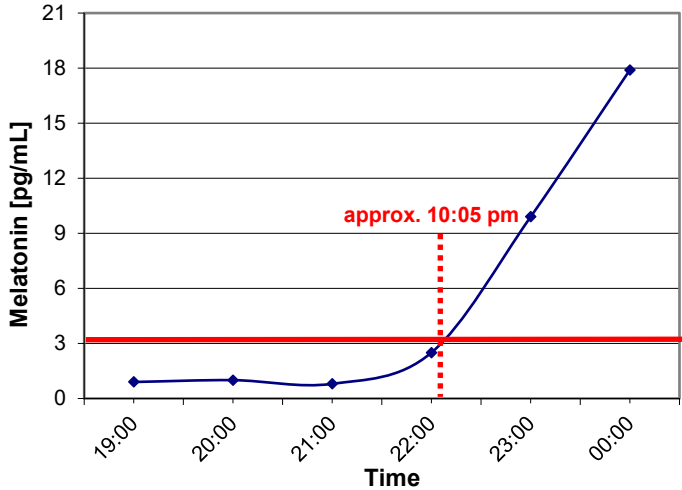
The melatonin results of the six (to eight) time points shall be plotted on a time axis: on the X-axis the six (to eight) time points and on the Y-axis the calculated concentrations of Melatonin in pg/mL. The melatonin curve can be represented by a point-to-point fitting or another curve fitting algorithm. Danilenko et al proposes a “hockey-stick” fitting method (7). The Dim-Light Melatonin Onset (DLMO) is defined as the time at which individual salivary melatonin concentration reaches a value of 2 to 4 pg/mL depending on the melatonin immunoassay used (4). For the sake of easiness and comparability, we recommend to apply a fixed DLMO threshold at 3 pg/mL. However, due the individually very different absolute melatonin values observed, a dynamic definition of the DLMO may be used instead of a fixed threshold, e.g. the time point at which the first melatonin value is clearly above the baseline followed by the next value which should be even higher than the first value clearly above baseline. A more sophisticated dynamic model is proposed by Danilenko et al. (7).

Example:

Habitual subject's bed/sleep time is 11 pm (23:00).

Collection time	Melatonin [pg/ml]
19:00 (7 pm)	0.9
20:00 (8 pm)	1.0
21:00 (9 pm)	0.8
22:00 (10 pm)	2.5
23:00 (11 pm)	9.9
00:00 (12 pm)	17.9

Using a fixed threshold of 3 pg/mL in this example, the DLMO time can be estimated to be around 22:05 (10:05 pm):

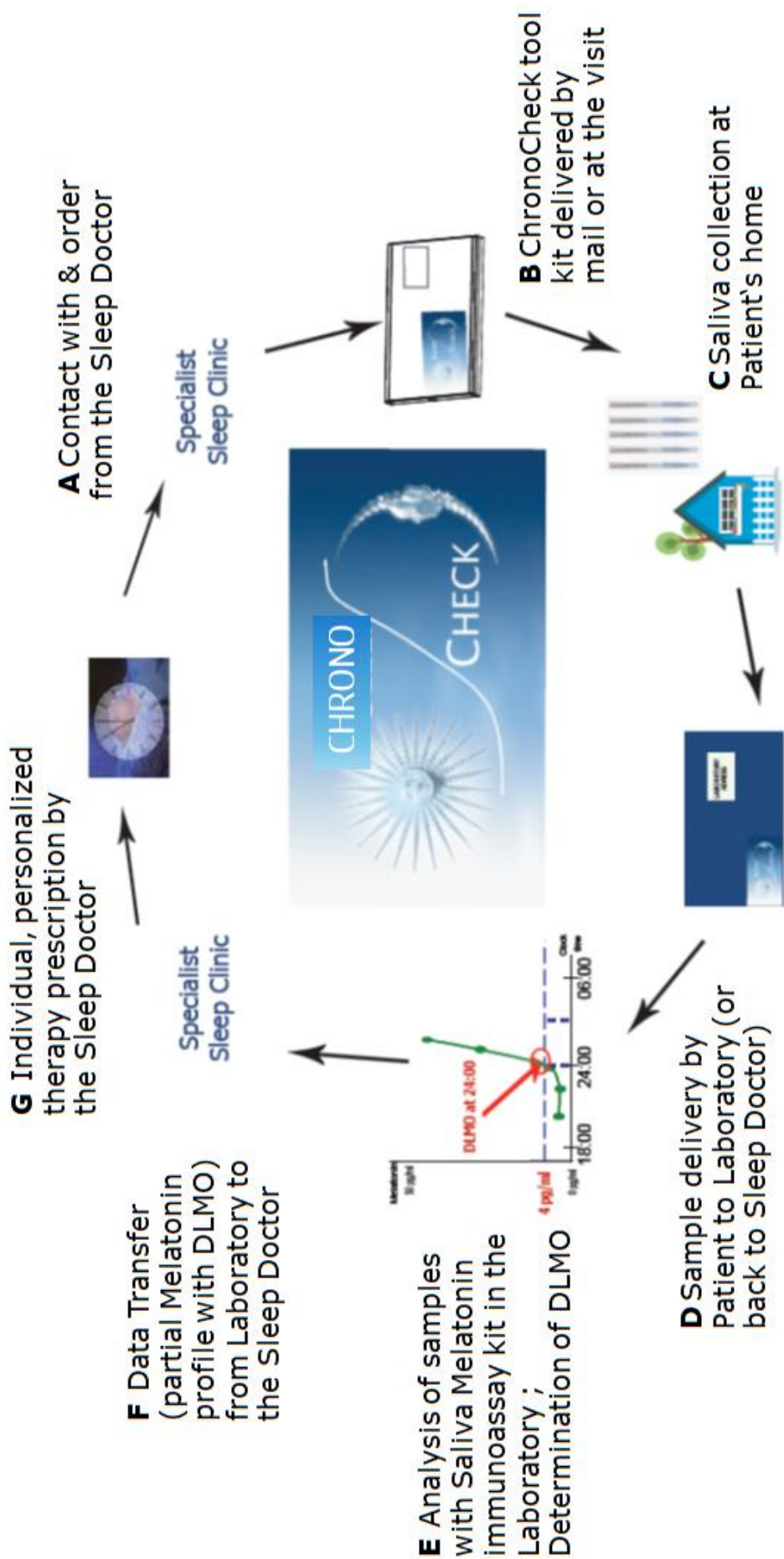


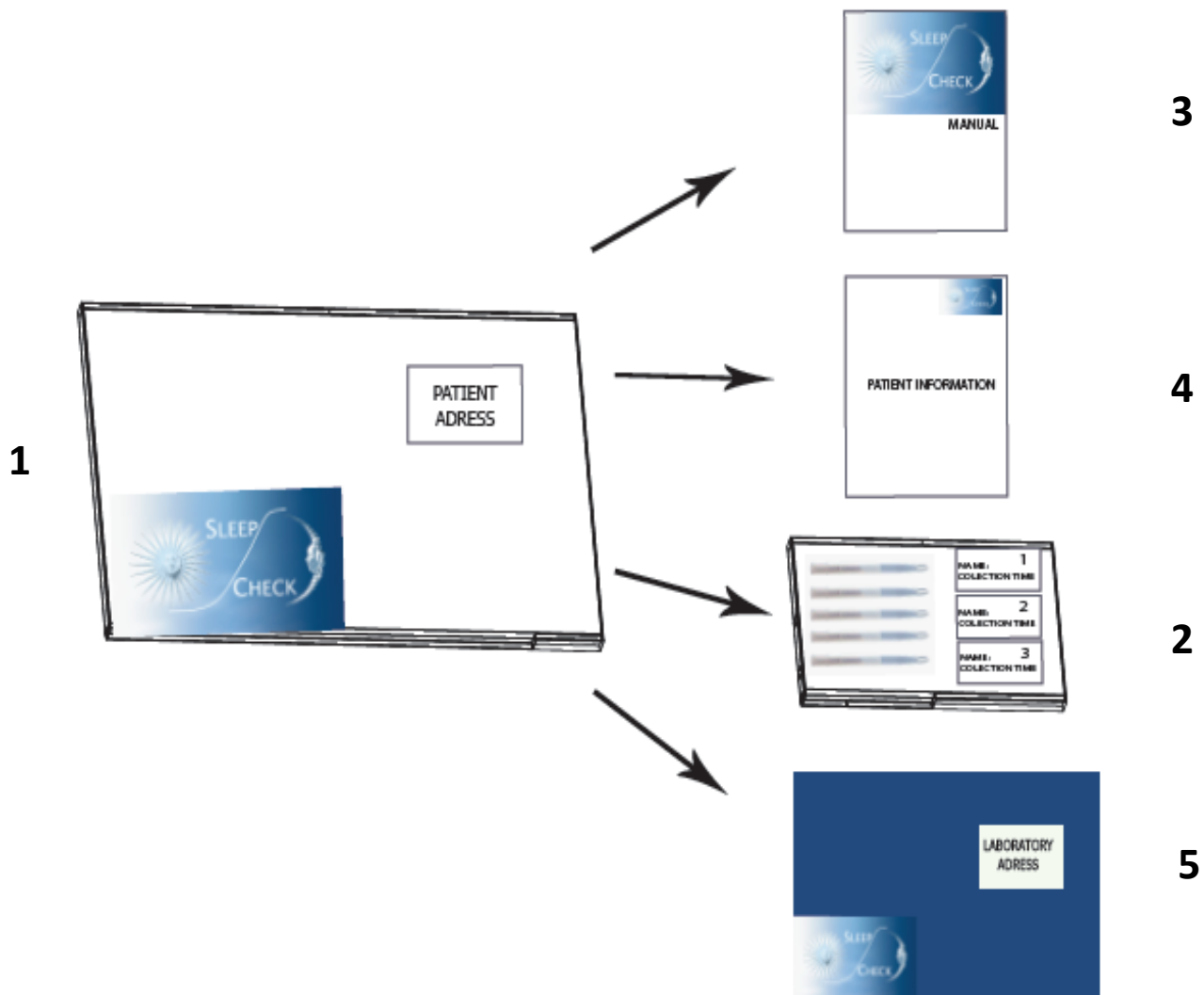
In some 10% of cases, the DLMO determination may not be successful with a 6-point partial melatonin profile:

- a) Concentrations of melatonin do not reach the pre-determined cut-off value (e.g. 3 pg/mL). These patients are either low melatonin secretors or their individual DLMO occurs after the last collection time point.
- b) All Melatonin levels are above pre-determined cut-off value (e.g. 3 pg/mL) for all six collection time points. Most probably, the patient's DLMO happened before the first collection point or the patient shows a profoundly shifted melatonin profile or the saliva samples may be contaminated due to improper handling during collection and/or immunoassay.

For both cases a) and b), it is recommended to collect either more samples before and/or after habitual sleep/bed time or to sample a 24-hours profile to determine the correct DLMO.

1. Voultsios A, *et al.* *Salivary melatonin as a circadian phase marker: validation and comparison to plasma melatonin.* J Biol Rhythms 12; 457-466 (1997)
2. Claustrat B, *et al.* *The basic physiology and pathophysiology of Melatonin Sleep.* Med Rev 9; 11-24 (2005)
3. Pandi-Perumal SR, *et al.* *Dim light melatonin onset (DLMO): A tool for the analysis of circadian phase in human sleep and chronobiological disorders.* Progr Neuro-Psychopharmacol Biol Psychiatry 31; 1–11 (2007)
4. Smits MG, *et al.* *Influence of Melatonin on Quality of Life in Patients with chronic fatigue and late Melatonin onset.* J Chronic Fatigue Syndr 10; 3-4 (2002)
5. Wirz-Justice A, *et al.* *Evening Melatonin and bright light administration induce additive phase shifts in dim light melatonin onset.* J Pineal Res 36; 192-194 (2004)
6. Nagtegaal JE, *et al.* *Effects of melatonin on the quality of life in patients with delayed sleep phase syndrome.* J Psychosom Res. 48; 45-50 (2000)
7. Danilenko KV, *et al.* *The hockey-stick method to estimate evening dim light melatonin onset (DLMO) in humans.* Chronobiol Int 31; 349-355 (2014)





- 1: White paper air-cushion envelope
- 2: Safety box containing 8 Salivettes®
- 3: Patient Manual (instructions for saliva sampling)
- 4: Patient data form
- 5: Plastic safety envelope

(Remark: The symbols/artwork *Sleep Check* may still appear on some items due to using up stock materials previously been used for the same application as this new, called *ChronoCheck* since its renovation and re-launch in 2025)

APPENDIX IV: SYMBOLS

Symbol	Explanation
	Use By Verwendbar bis Utiliser jusqu'au Utilizzare entro Fecha de caducidad
	Catalogue number Bestellnummer Référence du catalogue Numero di catalogo Número de catálogo
	Batch code Chargenbezeichnung Code du lot Codice del lotto Codigo de lote

Symbol	Explanation
	Contains sufficient for <n> tests Ausreichend für "n" Ansätze Contenu suffisant pour „n“ tests Contenuto sufficiente per „n“ saggi Contenido suficiente para <n> ensayos
	Consult Instructions for Use Gebrauchsanweisung beachten Consulter le mode d'emploi Consultare le istruzioni per l'uso Consulte las instrucciones de uso
	Temperature limitation Zulässiger Temperaturbereich Limites de température Limiti di temperatura Limite de temperatura