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RealStar®
VZV PCR Kit 1.2

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RealStar®

VZV PCR Kit 1.2

For use with

SmartCycler® II (Cepheid)

LightCycler® 1.2/1.5/2.0 Instruments (Roche)

CE

IVD For *in vitro* diagnostic use

REF Product No.: 071212

Σ 48 rxns

Store at -25°C ... -15°C

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altona Diagnostics GmbH • Mörkenstraße 12 • D-22767 Hamburg

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Content

1. Intended Use

The RealStar® VZV PCR Kit 1.2 is an *in vitro* diagnostic test, based on real-time PCR technology, for the detection and quantification of Varicella-Zoster Virus (VZV) specific DNA.

2. Kit Components

Lid Color	Blue	Purple	Green	Red	White
Component	Master A	Master B	Internal Control	Quantification Standard*	PCR grade Water
Number of Vials	4	4	1	4	1
Volume [µl/Vial]	60	120	1000	250	500

*The RealStar® VZV PCR Kit 1.2 contains four different Quantification Standards (QS1-QS4)

3. Storage

- The RealStar® VZV PCR Kit 1.2 is shipped on dry ice. The components of the kit should arrive frozen. If one or more components are not frozen upon receipt, or if tubes have been compromised during shipment, contact altona Diagnostics GmbH for assistance.
- All components should be stored at -20°C upon arrival.
- Repeated thawing and freezing of Master reagents (more than twice) should be avoided, as this might affect the performance of the assay. The reagents should be frozen in aliquots, if they are to be used intermittently.
- Storage at +4°C should not exceed a period of two hours.
- Protect Master A and Master B from light.

4. Material and Devices required but not provided

- Appropriate real-time PCR instrument (chapter 6. Product Description)
- Appropriate nucleic acid extraction system or kit
- Desktop centrifuge with a rotor for 2 ml reaction tubes
- Minicentrifuge with a rotor for Cepheid reaction tubes
- Vortex mixer
- LightCycler® capillaries with corresponding closing material
- Cepheid reaction tubes for the SmartCycler® II
- Pipettes (adjustable)
- Pipette tips with filters (disposable)
- Powder-free gloves (disposable)

NOTE

⚠ ***Please ensure that instruments have been installed, calibrated, checked and maintained according to the manufacturer´s instructions and recommendations.***

5. Background Information

Varizella Zoster Virus (VZV) is a member of the family Herpesviridae and, along with HSV-1 and HSV-2, is classified as an *alpha* Herpesvirus. VZV has a linear double stranded DNA genome consisting of approx. 125 kbp.

VZV spreads by droplet inhalation or direct contact with infectious lesions. More than 90% of adults have antibody to VZV. The virus causes two different clinical manifestations: varicella (chickenpox) and zoster (shingles). Varizella is the primary infection with VZV and is highly contagious. Varizella occurs most frequently in children. In contrast to primary infections with the other herpes group viruses, which are usually asymptomatic, varizella is usually clinically apparent and characterized by a generalized vesicular exanthem often accompanied by fever. Zoster (shingles) is a secondary infection due to reactivation of latent VZV in sensory ganglia. Zoster usually occurs in adults or immunocompromised patients and consists of a painful, circumscribed eruption of vesicular lesions with accompanying inflammation of associated dorsal root or cranial nerve sensory ganglia.

6. Product Description

The RealStar® VZV PCR Kit 1.2 is an *in vitro* diagnostic test, based on real-time PCR technology, for the detection and quantification of VZV specific DNA. The assay includes a heterologous amplification system (Internal Control) to identify possible PCR inhibition and to confirm the integrity of the reagents of the kit.

The test is based on real-time PCR technology, utilizing polymerase chain reaction (PCR) for the amplification of specific target sequences and target specific probes for the detection of the amplified DNA. The probes are labelled with fluorescent reporter and quencher dyes.

Probes specific for VZV DNA are labelled with the fluorophore FAM. The probe specific for the Internal Control (IC) is labelled with a fluorophore showing the same characteristics as Cy3. Using probes linked to distinguishable dyes enables the parallel detection of VZV specific DNA and the Internal Control in corresponding detector channels of the real-time PCR instrument.

The test consists of two processes in a single tube assay:

- PCR amplification of target DNA and Internal Control
- Simultaneous detection of PCR amplicons by fluorescent dye labelled probes

The RealStar® VZV PCR Kit 1.2 was developed and validated to be used with the following real-time PCR instruments:

- SmartCycler® II (Cepheid)
- LightCycler® 1.2/1.5/2.0 Instruments (Roche)

The RealStar® VZV PCR Kit 1.2 consists of:

- Two Master reagents (Master A and Master B)
- Template Internal Control (IC)
- Four Quantification Standards (QS1 – QS4)
- PCR grade water

Master A and Master B reagents contain all components (buffer, enzymes, primers and probes) to allow PCR mediated amplification and target detection of VZV specific DNA and Internal Control in one reaction setup.

The Quantification Standards contain standardized concentrations of VZV specific DNA. The Quantification Standards can be used individually as positive controls or together to generate a **standard curve,** which can be used to determine the concentration of VZV in the sample.

The following concentrations are used:

Quantification Standards	Concentration [copies/µl]
QS1	1.00E+04
QS2	1.00E+03
QS3	1.00E+02
QS4	1.00E+01

7. Warnings and Precautions

- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.
- Specimens should always be treated as if infectious and/or biohazardous in accordance with safe laboratory procedures.
- Wear protective disposable powder-free gloves, a laboratory coat and eye protection when handling specimens.
- Avoid microbial and nuclease (DNase/RNase) contamination of the specimen and the components of the kit.
- Always use DNase/RNase-free disposable pipette tips with aerosol barriers.
- Always wear protective disposable powder-free gloves when handling kit components.
- Use separated and segregated working areas for (i) specimen preparation, (ii) reaction set-up and (iii) amplification/detection activities. Workflow in the laboratory should proceed in unidirectional manner. Always wear disposable gloves in each area and change them before entering different areas.
- Dedicate supplies and equipment to the separate working areas and do not move them from one area to another.
- Store positive and/or potentially positive material separated from all other components of the kit.
- Do not open the reaction tubes/capillaries post amplification, to avoid contamination with amplicons.
- Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations.
- Do not use components of the kit that have passed their expiration date.
- Discard sample and assay waste according to your local safety regulations.

8. Instructions for Use

8.1 Sample Preparation

Extracted DNA is the starting material for the RealStar® VZV PCR Kit 1.2. The quality of the extracted DNA has a profound impact on the performance of the entire test system. It has to be ensured that the system used for nucleic acid extraction is compatible with real-time PCR technology.

The following nucleic acid extraction systems and kits are recommended:

- VERSANT™ Molecular System SP (Siemens)
- HighPure® Viral Nucleic Acid Kit (Roche)
- QIAamp® DNA Mini Kit (QIAGEN)

If using a spin column based sample preparation procedure including washing buffers containing ethanol, an additional centrifugation step for 10 min at approximately 17000 x g (~ 13000 rpm), using a new collection tube, prior to the elution of the nucleic acid is highly recommended.

NOTE

⚠ ***The use of carrier RNA is crucial for extraction efficiency and stability of the extracted nucleic acid.***

⚠ ***Ethanol is a strong inhibitor in real-time PCR. If your sample preparation system is using washing buffers containing ethanol, you need to make sure to eliminate any traces of ethanol prior to elution of the nucleic acid.***

For additional information and technical support regarding pre-treatment and sample preparation please contact our Technical Support:

e-mail: **support@altona-diagnostics.com**
phone: **+49-(0)40-5480676-0**

8.2 Master Mix Setup

All reagents and samples should be thawed completely, mixed (by pipetting or gentle vortexing) and centrifuged briefly before use.

The RealStar® VZV PCR Kit 1.2 contains a heterologous Internal Control (IC), which can either be used as a PCR inhibition control or as a control of the sample preparation procedure (nucleic acid extraction) and as a PCR inhibition control.

- If the IC is used as a PCR inhibition control, but not as a control for the sample preparation procedure, the Master Mix is set up according to the following pipetting scheme:

Number of Reactions (rxns)	1	12
Master A	5 µl	60 µl
Master B	10 µl	120 µl
Internal Control	1 µl	12 µl
Volume Master Mix	**16 µl**	**192 µl**

- If the IC is used as a control for the sample preparation procedure and as a PCR inhibition control, the IC has to be added during the nucleic acid extraction procedure.

- No matter which method/system is used for nucleic acid extraction, the IC **must not** be added directly to the specimen. The IC should always be added to the specimen/lysis buffer mixture. The volume of the IC which has to be added depends always and only on the elution volume. It represents 10% of the elution volume. For instance, if the nucleic acid is going to be eluted in 60 µl of elution buffer or water, 6 µl of IC per sample must be added into the specimen/lysis buffer mixture.

NOTE

⚠ ***Never add the Internal Control directly to the specimen!***

- If the IC was added during the sample preparation procedure, the Master Mix is set up according to the following pipetting scheme:

Number of Reactions (rxns)	1	12
Master A	5 µl	60 µl
Master B	10 µl	120 µl
Volume Master Mix	**15 µl**	**180 µl**

8.3 Reaction Setup

- Pipette 15 µl of the Master Mix into each required LightCycler® capillary or reaction tube for the SmartCycler® II.
- Add 10 µl of the sample (eluate from the nucleic acid extraction) or 10 µl of the controls (Quantification Standard, Positive or Negative Control).
- Make sure that at least one Positive and one Negative Control are used per run.
- For quantification purposes all Quantification Standards (QS1 to QS4) should be used.
- Thoroughly mix the samples and controls with the Master Mix by up and down pipetting.
- Close the capillaries or the tubes using appropriate lids.
- Centrifuge the LightCycler® capillaries or reaction tubes of the SmartCycler® II using an appropriate centrifuge for 30 seconds at approximately ~400 x g (~2000 rpm).

Reaction Setup	
Master Mix	15 µl
Sample or Control	10 µl
Total Volume	**25 µl**

9. Programming the Real-Time PCR Instruments

For basic information regarding the setup and programming of the different real-time PCR instruments, please refer to the manual of the respective instrument.
For detailed programming instructions regarding the use of the RealStar® VZV PCR Kit 1.2 on specific real-time PCR instruments please contact our Technical Support.

9.1 Settings

- Define the following settings:

Settings	
Reaction Volume	25 µl*
Ramp Rate	Default

*The reaction volume has to be defined as 20 µl, if using a LightCycler® 2.0 Instrument (Roche).

9.2 Fluorescent Detectors (Dyes)

- Define the fluorescent detectors (dyes):

	Detection Channel		
Detection	**LightCycler® 1.2/1.5**	**LightCycler® 2.0**	**SmartCycler® II**
VZV specific DNA	F1	530	FAM
Internal Control	F2	610	Cy3

NOTE

> ⚠ ***For accurate data analysis on the LightCycler® instruments a specific Color Compensation File might be needed. Please contact altona Diagnostics GmbH for assistance.***
>
> ⚠ ***If using the LightCycler® 2.0 Instrument, only the detection channels 530 and 610 should be activated for color compensation.***

9.3 Temperature Profile and Dye Acquisition

- Define the temperature profile and dye acquisition:

	Analysis Mode	Cycle Repeats	Acquisition	Temperature	Time
Denaturation	None	1	-	95 °C	2:00 min
Amplification	Quantification	45	None	95 °C	0:05 min
			Single	60 °C	0:30 min
			None	72 °C	0:10 min
Cooling	None	1	-	40 °C	0:30 min

10. Data Analysis

For basic information regarding data analysis on specific real-time PCR instruments, please refer to the manual of the respective instrument.
For detailed instructions regarding data analysis of the RealStar® VZV PCR Kit 1.2 on different real-time PCR instruments please contact our Technical Support.

10.1 Validity of Diagnostic Test Runs

10.1.1 Valid Diagnostic Test Run (qualitative)

For a **valid** diagnostic test run (**qualitative**), the following control conditions must be met:

Control ID	FAM /F1/530 Detection Channel	Cy3/F2/610 Detection Channel
Positive Control (QS)	POSITIVE	POSITIVE
Negative Control	NEGATIVE	POSITIVE

10.1.2 Invalid Diagnostic Test Run (qualitative)

A **qualitative** diagnostic test run is **invalid**, (i) if the run has not been completed or (ii) if any of the control conditions for a **valid** diagnostic test run are not met.

In case of an **invalid** diagnostic test run, repeat testing by using the remaining purified nucleic acids or start from the original samples again.

10.1.3 Valid Diagnostic Test Run (quantitative)

For the validity of a **quantitative** diagnostic test run, all control conditions of a **valid qualitative** diagnostic test run must be met [chapter 10.1.1 Valid Diagnostic Test Run (qualitative)]. Furthermore, for accurate quantification results a valid **standard curve** has to be generated. For a **valid quantitative** diagnostic test run, the following control parameter values of the standard curve should be achieved:

Control Parameter	Valid Value
Slope	- 3.00 / - 3.74
PCR Efficiency	85 % / 115 %
R square (R^2)	> 0.98

NOTE

⚠ ***Not all parameters are displayed by the software of the different real-time PCR instruments. For detailed information, please refer to the manual of the respective instrument.***

10.1.4 Invalid Diagnostic Test Run (quantitative)

A **quantitative** diagnostic test run is **invalid**, (i) if the run has not been completed or (ii) if any of the control conditions for a **valid** diagnostic test run are not met.

In case of an **invalid** diagnostic test run, repeat testing by using the remaining purified nucleic acids or start from the original samples again.

10.2 Interpretation of Results

10.2.1 Qualitative Analysis

Sample ID	FAM/F1/530 Detection Channel	Cy3/F2/610 Detection Channel	Result Interpretation
A	POSITIVE	POSITIVE*	VZV specific DNA detected.
B	NEGATIVE	POSITIVE	VZV specific DNA not detected. Sample does not contain detectable amounts of VZV specific DNA.
C	NEGATIVE	NEGATIVE	PCR inhibition or reagent failure. Repeat testing from original sample or collect and test a new sample.

* Detection of the Internal Control in the Cy3/F2/610 detection channel is not required for positive results in the FAM/F1/530 detection channel. High VZV loads in the sample can lead to reduced or absent Internal Control signals.

10.2.2 Quantitative Analysis

The RealStar® VZV PCR Kit 1.2 provides four Quantification Standards (QS). In order to generate a **standard curve** in quantitative analysis, these have to be defined as **standards** with appropriate concentrations (chapter 6. Product Description). Using **standards** of known concentrations a standard curve for quantitative analysis can be generated.

$C_t = m \cdot \log (N_0) + b$

C_t = Threshold Cycle
m = Slope
N_o = Initial Concentration
b = Intercept

Derived from the standard curve positive samples of unknown concentrations can be quantified.

$N_0 = 10^{(Ct-b)/m}$

A B

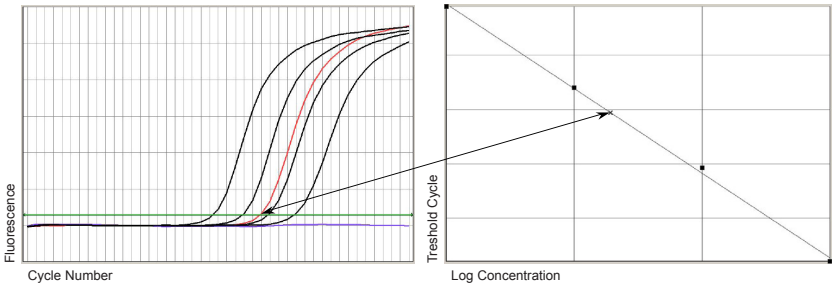

Figure 1: Quantification Standards (black), a positive (red) and a negative sample (blue) displayed in the Amplification Plot (**A**) and Standard Curve analysis (**B**).

NOTE

⚠ ***The concentration of the "Sample" is displayed in copies/µl and refers to the concentration in the eluate.***

To determine the **viral load of the original sample**, the following formula has to be applied:

$$\text{Viral load (Sample) [copies/ml]} = \frac{\text{Volume (Eluate) [µl] x Viral load (Eluate) [copies/µl]}}{\text{Sample Input [ml]}}$$

11. Performance Evaluation

Performance evaluation of the RealStar® VZV PCR Kit 1.2 was done, using quantified VZV specific DNA isolated from VZV strain ELLEN (ATCC® Number: VR-1367).

11.1 Analytical Sensitivity

The analytical sensitivity of the RealStar® VZV PCR Kit 1.2 is defined as the concentration (copies per µl of the eluate) of VZV specific DNA molecules that can be detected with a positivity rate of ≥ 95 %. The analytical sensitivity was determined by analysis of dilution series of quantified VZV DNA.

Table 1: PCR results used for the calculation of the analytical sensitivity of the RealStar® VZV PCR Kit 1.2

Input Conc. [copies/µl]	Number of Replicates	Number of Positives	Hit Rate [%]
3.160	12	12	100
1.000	12	12	100
0.316	12	11	92
0.100	12	8	67
0.032	12	4	33
0.010	12	0	0
0.003	12	0	0
NTC	12	0	0

Analytical sensitivity of the RealStar® VZV PCR Kit 1.2, determined by Probit analysis, is 0.34 copies/µl [95 % confidence interval (CI): 0.18 – 1.22 copies/µl].

11.2 Analytical Specificity

The analytical specificity of the RealStar® VZV PCR Kit 1.2 is ensured by the thorough selection of the oligonucleotides (primers and probes). The oligonucleotides were checked by sequence comparison analysis against public available sequences to ensure that all relevant VZV genotypes will be detected.
The analytical specificity of the RealStar® VZV PCR Kit 1.2 was evaluated by testing a panel of genomic DNA/RNA extracted from other herpesviruses or other pathogens significant in immunocompromised patients.

Table 2: Organisms tested to demonstrate the analytical specificity of the RealStar® VZV PCR Kit 1.2

Cy3/F2/610	RealStar® VZV PCR Kit 1.2	
Organisms	**FAM/F1/530 Channel (VZV)**	**Cy3/F2/610 Channel (Internal Control)**
Herpes Simplex Virus 1	Negative	Valid
Herpes Simplex Virus 2	Negative	Valid
Varicella-Zoster Virus	Positive	Valid
Epstein-Barr Virus	Negative	Valid
Cytomegalovirus	Negative	Valid
Human Herpesvirus 6 (A, B)	Negative	Valid
Human Herpesvirus 7	Negative	Valid
Human Herpesvirus 8	Negative	Valid
BK Virus	Negative	Valid
JC Virus	Negative	Valid
Parvovirus B19	Negative	Valid
Hepatitis B Virus	Negative	Valid
Hepatitis C Virus	Negative	Valid
Human Immunodeficiency Virus 1	Negative	Valid

The RealStar® VZV PCR Kit 1.2 did not cross-react with any of the specified organisms.

11.3 Linear Range

The linear range of the RealStar® VZV PCR Kit 1.2 was evaluated by analysing a logarithmic dilution series of VZV specific DNA using concentrations ranging from 1E+08 to 1E+00 copies/µl. At least eight replicates per dilution were analysed.

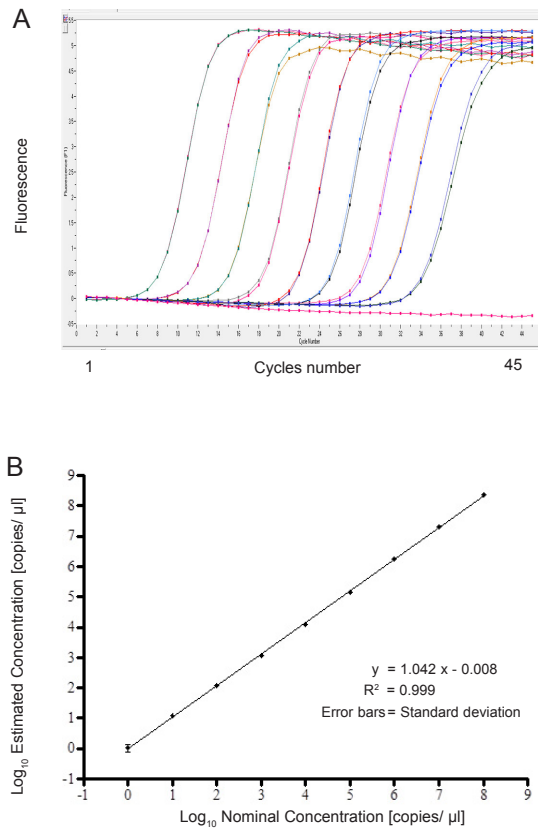

Figure 2: Amplification curves [A] and linear regression [B] of analysed dilution series of VZV specific DNA.

The linear range of the RealStar® VZV PCR Kit 1.2 extends over an interval of at least **eight** orders of magnitude.

11.4 Precision

Precision data for the RealStar® VZV PCR Kit 1.2 were determined as intra-assay variability (variability within one experiment), inter-assay variability (variability between different experiments) and inter-lot variability (variability between different production lots).

Variability data are expressed in terms of standard deviation, variance and coefficient of variation. The data are based on quantification analysis of defined concentrations of VZV specific DNA and on threshold cycle (C_t) value in terms of the Internal Control. At least six replicates per sample were analysed for intra-assay, inter-assay and inter-lot variability. Total variance was calculated by combining the three analyses.

Table 3: Precision data of VZV specific system of the RealStar® VZV PCR Kit 1.2

VZV specific System	Average Conc. (copies/µl)	Standard Deviation	Variance	Coefficient of Variation (%)
Intra-Assay Variability	132.87	2.64	6.96	1.99
Inter-Assay Variability	135.87	10.74	115.33	7.90
Inter-Lot Variability	133.01	12.70	161.41	9.55
Total Variance	134.96	13.37	178.74	9.91

Table 4: Precision data for the Internal Control of the RealStar® VZV PCR Kit 1.2 (C_t-values)

Internal Control	Average Threshold Cycle (C_t)	Standard Deviation	Variance	Coefficient of Variation (%)
Intra-Assay Variability	25.27	0.09	0.01	0.35
Inter-Assay Variability	25.48	0.28	0.08	1.10
Inter-Lot Variability	25.53	0.29	0.08	1.12
Total Variance	25.59	0.27	0.08	1.07

11.5 Repeatability

Specificity, sensitivity and accuracy of quantification of the RealStar® VZV PCR Kit 1.2 were evaluated by analysing established proficiency panels for VZV. To ensure repeatability of the RealStar® VZV PCR Kit 1.2, specificity and sensitivity are evaluated by analysing established proficiency panels for VZV as well as characterized diagnostic samples on a regular basis.

12. Limitations and Precautions

- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR and in *in vitro* diagnostic procedures.
- Good laboratory practice is essential for proper performance of this assay. Extreme care should be taken to preserve the purity of the components of the kit and reaction setups. All reagents should be closely monitored for impurity and contamination. Any suspicious reagents should be discarded.
- Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test.
- This assay is not to be used on the specimen directly. Appropriate nucleic acid extraction methods have to be conducted prior to using this assay.
- The presence of PCR inhibitors may cause false negative or invalid results.
- Potential mutations within the target regions of the VZV genome covered by the primers and/or probes used in the kit may result in failure to detect the presence of the pathogen.
- As with any diagnostic test, results of the RealStar® VZV PCR Kit 1.2 should be interpreted in consideration of all clinical and laboratory findings.

13. Quality Control

In accordance with the altona Diagnostics GmbH ISO EN 13485-certified Quality Management System, each lot of RealStar® VZV PCR Kit 1.2 is tested against predetermined specifications to ensure consistent product quality.

14. Technical Assistance

For customer support, please contact our Technical Support:

e-mail: **support@altona-diagnostics.com**
phone: **+49-(0)40-5480676-0**

15. Trademarks and Disclaimers

RealStar® (altona Diagnostics GmbH); LightCycler®, HighPure® (Roche); QIAamp® (QIAGEN); SmartCycler® (Cepheid); VERSANT™ (Siemens).

Registered names, trademarks, etc. used in this document, even if not specifically marked as such, are not to be considered unprotected by law.

The RealStar® VZV PCR Kit 1.2 is a CE-marked diagnostic kit according to the European *in vitro* diagnostic directive 98/79/EC.

Not available in all countries.

16. Explanation of Symbols

In vitro diagnostic medical device

Product number

Batch code

Contains sufficient for “n” tests/reactions (rxns)

Temperature limitation

Version

Use until

Caution

Consult instructions for use

Manufacturer

Notes