EGFR Mutation Test kit (RUO)

Detection of 44 mutations in Exons 18-21
Instruction for Use

GENECAST, Co., Ltd.
T-10009 Garden Five Techno, 66 Chungmin-ro, Songpa-gu, Seoul, South Korea ZIP 05838

Tel: +82 2 2157 3150 | Fax: +82 2 2157 3152
E-mail: sales@genecast.com
Website: http://www.genecast.com

Background

Lung cancer is the leading cause of cancer-related mortality in the United States and lung cancer makes up about 14% of all new cancer diagnoses. There are two main classifications of lung cancer; NSCLC (Non-Small Cell Lung Cancer) and SCLC (Small Cell Lung Cancer). These two types are treated differently. NSCLC is the most common type of lung cancer, accounting for 80% to 85% of all lung cancer diagnoses. The only screening modality for early detection that has been shown to alter mortality is low-dose helical CT scanning in patients considered at high risk for developing lung cancer. The 5-year survival rate for all people with all types of lung cancer is 18%. The survival rates depend on several factors, including the subtype of lung cancer and the stage of disease.

Non-small cell lung cancer (NSCLC) is the most common type of lung cancer, accounting for 80% to 85% of all lung cancer diagnoses. Patients with epidermal growth factor receptor (EGFR) gene mutations are known to exhibit very high drug responses or resistances against EGFR Tyrosine Kinase Inhibitor (TKI) such as Gefitinib (Iressa, AstraZeneca), Erlotinib (Tarceba, Roche) and Osimertinib (Tagrisso, AstraZeneca). G719X, S768I, Ex19 deletion, L858R and L861Q mutations are associated with susceptibility to EGFR TKIs, while T790M and most Ex20 insertion mutations are associated with reduced EGFR TKI responses. Early detection of EGFR gene mutations in lung cancer patients allows to predict drug response before treatment.

However, our understanding of an individual patient's cancer is often limited by tumor accessibility because of the high risk and invasive nature of current tissue biopsy procedures. "Liquid biopsy", the analysis of circulating tumor DNA (ctDNA), offers a new source of cancer-derived materials that may reflect the status of the disease better and thereby contribute to more personalized treatment. In addition, it is critical to develop more sensitive technologies for detection of mutation.

The ADPS™ EGFR Mutation Test kit (RUO) is a qualitative PCR assay designed to detect 44 somatic mutations in exon 18-21. This kit can detect up to 0.01% of the EGFR mutations in DNA extracted from FFPET (Formalin-fixed paraffin-embedded tissue) or plasma and enables qualitative detection.

Intended Use

The ADPS™ EGFR Mutation Test kit (RUO) is a real–time PCR assay for qualitative detection of 44 somatic mutations in exons 18-21 of the EGFR gene. DNA extracted from formalin-fixed paraffin-embedded samples of tissue biopsy or circulating free DNA extracted from liquid biopsy could be used. The kit is for Research Use Only (RUO), and intended to be used by trained professionals in a laboratory environment.

Principles of the Procedure

The kit adopts Allele-specific PCR (AS-PCR) and real-time PCR technologies, which comprises specific primers and fluorescent probes to detect EGFR mutations in cfDNA from human plasma samples. During the nucleic acid amplification, the targeted mutant DNA is matched with the bases at 3' end of the primer,

amplified selectively and efficiently, then the mutant amplicon is detected by fluorescent probes labeled with FAM or CAL Fluor Orange 560 (CFO560). While the wild-type DNA cannot be matched with specific primers, there is no amplification occurs.

The kit is composed of EGFR Master Mixture 1-4, ADPS™ smart DNA Polymerase, EGFR Positive Control and Nuclease-free water.

1) The contents in EGFR Master Mixture 1-4 formed a mutation detection system and an internal control system. The mutation detection system includes specific primers and FAM or CAL Fluor Orange 560-labeled probes for designated EGFR mutations. The internal control system contains the primers and Quasar 670-labeled probe for a EGFR region of genomic DNA without known mutations and polymorphism, to check the presence of inhibitors and monitor the accuracy of experimental operation.

2) The EGFR Positive Control contains recombinant plasmid DNA that carries fragments of the EGFR gene with each mutation.

3) The ADPS™ smart DNA Polymerase contains ADPS™ DNA polymerase for PCR amplification

Kit Contents

This kit contains the following materials (see Table 1):

Table 1. Kit Contents (for 24 tests)

Contents	Main Ingredient	Quantity
EGFR Master Mixture 1	Buffers with dNTPs, Primers/Probes for EGFR targets and IC	240 µL/tube ×1
EGFR Master Mixture 2	Buffers with dNTPs, Primers/Probes for EGFR targets and IC	240 µL/tube ×1
EGFR Master Mixture 3	Buffers with dNTPs, Primers/Probes for EGFR targets and IC	240 µL/tube ×1
EGFR Master Mixture 4	Buffers with dNTPs, Primers/Probes for EGFR targets and IC	240 µL/tube ×1
ADPS™ smart DNA Polymerase	ADPS™ smart DNA Polymerase	60 µL/tube ×1
EGFR Positive Control	Recombinant plasmid blends harboring each EGFR mutation	160 µL/tube ×1
Nuclease-free water	PCR grade water	1.5 mL/tube ×1

The EGFR Master Mixture 1-4 contains all reagents except ADPS™ smart DNA polymerase that is supplied in a separate tube and has to be added before the assay. The detailed detection information is listed in Table 2.

Table 2. Detection Information

Reagent	Fluorescence Signal		
	FAM	CAL Fluor Orange 560*	Quasar 670*
EGFR Master Mixture 1	S768I	Ex19del	IC**
EGFR Master Mixture 2	T790M		IC
EGFR Master Mixture 3	L861Q	G719X	IC
EGFR Master Mixture 4	Ex20Ins	L858R	IC

*Alternative dye: CAL Fluor Orange 560 (VIC/HEX/JOE), Quasar 670 (CY5)

**IC: Internal control for PCR

Storage and Stability

The kit requires shipment on frozen ice packs. All contents of the kit should be stored immediately upon receipt at -20±5°C and protected from light.

The shelf-life of the kit is 18 months, and 90 days of opened reagents for the kit. EGFR Master Mixture1-4 and reaction Master Mixtures should be protected from prolonged exposure to light. Reaction Master Mixtures must be stored at 2ºC to 8ºC in the dark. The prepared samples and controls must be added within 1 hour of preparation of the reaction Master Mixtures. Amplification must be started within 1 hour from the time that the processed samples and controls are added to the reaction Master Mixtures.

The recommend maximum freeze-thaw cycle is five cycles.

Additional Reagents and Equipment Required but Not Supplied

1) Compatible PCR instruments: AB 7500 / 7500 FAST, QuantStudio 5 (Applied Biosystems), CFX96/384 Real-time system (Bio-rad).
2) DNA Extraction kit for FFPET or plasma. (Recommended : GeneRead DNA FFPE kit, QIAamp MinElute ccfDNA kit)
3) Spectrophotometer for measuring DNA concentration.
4) Mini centrifuge with rotor for centrifuge tubes.
5) Mini centrifuge with rotor for PCR tubes.
6) Nuclease-free centrifuge tubes.
7) Nuclease-free PCR tubes and caps.
8) Adjustable pipettors and filtered pipette tips for handling DNA.
9) Tube racks.
10) Disposable powder-free gloves.

Precautions and Handling Requirements (For research use only)

Precautions

- DO NOT use for *in-vitro* Diagnostic (IVD).
- Please read the instruction carefully and become familiar with all components of the kit prior to use, and strictly follow the instruction during operation.
- Please check the compatible real-time PCR instruments prior to use.
- DO NOT use the kit or any kit component after their expiry date.
- DO NOT use any other reagents from different lots in the tests.
- DO NOT use any other reagent in the other test kits.

Safety Information

- Handle all specimens and components of the kit as potentially infectious material using safe laboratory procedures.
- Only trained professionals can use this kit. Please wear suitable lab coat and disposable gloves while handling the reagents.
- Avoid skin, eyes and mucous membranes contact with the chemicals. In case of contact, flush with water immediately.
- DO NOT pipet by mouth.

Decontamination and Disposal

- The kit contains positive control; strictly distinguish the positive control from other reagents to avoid contamination which may cause false positive.
- PCR amplification is extremely sensitive to cross-contamination. The flow of tubes, racks, pipets and other materials used should be from pre-amplification to post-amplification, and never backwards.
- Gloves should be worn and changed frequently when handling samples and reagents to prevent contamination.
- Using separate, dedicated pipettes and filtered pipette tips when handling samples and reagents to prevent exogenous DNA contamination to the reagents.
- Please pack the post-amplification tubes with two disposable gloves and discard properly. DO NOT open the post- amplification PCR tubes.
- All disposable materials are for one time use. DO NOT reuse.
- The unused reagents, used kit, and waste must be disposed of properly.

Cleaning

- After the experiment, wipe down the work area, spray down the pipettes and equipment with 75% ethanol or 10% hypochlorous acid solution.

Instrument Setup

- Setup the reaction volume as 20 μL.
- For AB 7500 / 7500 FAST, QuantStudio 5, CFX384 Real-time system, please set up as follows: Reporter Dye: FAM, CY5; Quencher Dye: NFQ-MGB; Passive Reference dye: ROX.
- Refer to the real-time PCR instrument operator's manual for detailed instructions.
- We recommend that all PCR instruments in use should be conducted fluorescence calibration once a year.

Assay Procedure

1. DNA Extraction

The specimen material must be human genomic DNA extracted from FFPE tissue or circulating DNA extracted from the plasma sample. DNA extraction reagents are not included in the kit. Before DNA extraction, it's essential to use standard pathology methodology to ensure tumor sample quality. Carry out the DNA extraction according to the instructions of DNA extraction kit.

Tumor samples are non-homogeneous, may also contain non-tumor tissue. Data from different tissue sections of the same tumor may be inconsistent. DNA from non-tumor tissue would not be detected with *EGFR* mutations. It's better to use tumor tissue samples with more than 30% tumor cells.

The OD_{260}/OD_{280} value of extracted DNA from FFPE tissue should be between 1.7 ~ 2.1 (measured using the spectrophotometer). The storage conditions for extracted DNA from tissue and plasma used for PCR amplification is shown in Table 3. And the circulating DNA isolated from plasma should be used directly without dilution.

The recommended sample DNA amount is up to 99 ng/reaction (~30,000 copies of normal genomic DNA).

Table 3. Recommended Conditions for Sample and Extracted DNA

	≤ -70°C	**-15°C to -25°C**	**2°C to 8°C**	**15°C to 30°C**
FFPE	≤ 3 years			
Plasma	Up to 12 months		Up to 3 days	
Extracted DNA		Up to 2 freeze thaws over 60 days	Up to 21 days	Up to 7 days

Note:

- *The FFPE tissue should be handled and stored properly, and the storage time should preferably be less than 3 years.*

- *The plasma sample should be derived from EDTA anti-coagulated peripheral whole blood. PAXgene Blood ccfDNA Tube is recommended. The PAXgene Blood ccfDNA Tube allows collection of 10 ml whole blood into a closed, evacuated system.*
- *The extracted DNA should be used immediately, if not, it should be stored at -20±5 ℃ for no more than 2 months.*

2. Mutation Detection

1) Take the EGFR Master Mixture 1-4, EGFR Positive Control, Nuclease-free water and ADPS™ smart DNA Polymerase out of the kit from the freezer.

2) Thaw the EGFR Master Mixture 1-4, EGFR Positive Control, and Nuclease-free water at room temperature. When the reagents are completely thawed, vortex each tubes for 3 sec and centrifuge briefly to collect all liquid at the bottom of the tube.

3) Gently tap the ADPS™ smart DNA Polymerase and centrifuge briefly prior to use.

4) Prepare sufficient EGFR Reaction Mixture 1-4 containing ADPS™ smart DNA Polymerase, EGFR Master Mixture 1-4 in separate sterile centrifuge tube respectively according to the ratio in Table 4-1 to 4-2. Mix Reaction Master Mix thoroughly by vortexing for 3 sec and centrifuge briefly.

Table 4-1. *EGFR* Reaction Master Mixture for Mutation Detection

Content	Volume per test (μL)
EGFR Master Mixture	10.0
ADPS smart DNA polymerase	0.5
Sample	2.0~9.5
Nulclease-free water	0~7.5
Total volume	20.0

Table 4-2. *EGFR Reaction Master* Mixture for Positive control

Content	Volume per test (μL)
EGFR Master Mixture	10.0
ADPS smart DNA polymerase	0.5
EGFR Positive Control	5.0
Nulclease-free water	4.5
Total volume	20.0

Note:

- *Prepare a positive control experiment at the very end to prevent cross-contamination.*
- *Every PCR run must contain at least one PC (Positive control) and one NTC (No template control).*
- *The prepared mixtures should be used immediately, avoid prolonged storage.*
- *Due to the viscosity of the enzyme mix, pipet slowly to ensure all mix is completely dispensed from the tip.*
- *Pipet enzyme mix by placing the pipet tip just under the liquid surface to avoid the tip being coated in excess enzyme.*

5) Prepare two PCR tubes for each sample: Dispense 10.0 µL of *EGFR* Reaction Mixture to each PCR tube respectively. Then add 5.0 µL of each sample DNA to each sample tube and cap the PCR tubes.
6) Prepare one PCR tube for PC: Dispense 10.0 µL of *EGFR* Reaction Mixture to one PCR tube. Then add PC (5 µL) to sample tube and cap the PCR tube.
7) Add nuclease-free water to all PCR tubes upto 20.0 µL.
8) Briefly centrifuge the PCR strips to collect all liquid at the bottom of each PCR tube.
9) Place the PCR strip tubes into the real-time PCR instrument. A recommended plate layout is shown in Table 5.

Table 5. PCR Plate Layout (Example of 8 samples)

Well	1	2	3	4	5	6	7	8	9	10	11	12
A	NTC MMX1	Sample 1 MMX1	Sample 1 MMX2	Sample 1 MMX3	Sample 1 MMX4							
B	NTC MMX2	Sample 2 MMX1	Sample 2 MMX2	Sample 2 MMX3	Sample 2 MMX4							
C	NTC MMX3	Sample 3 MMX1	Sample 3 MMX2	Sample 3 MMX3	Sample 3 MMX4							
D	NTC MMX4	Sample 4 MMX1	Sample 4 MMX2	Sample 4 MMX3	Sample 4 MMX4							
E	PC MMX1	Sample 5 MMX1	Sample 5 MMX2	Sample 5 MMX3	Sample 5 MMX4							
F	PC MMX2	Sample 6 MMX1	Sample 6 MMX2	Sample 6 MMX3	Sample 6 MMX4							
G	PC MMX3	Sample 7 MMX1	Sample 7 MMX2	Sample 7 MMX3	Sample 7 MMX4							
H	PC MMX4	Sample 8 MMX1	Sample 8 MMX2	Sample 8 MMX3	Sample 8 MMX4							

10) Setup the PCR Protocol using the cycling parameters in Table 6.

Table 6. Cycling Parameters

Stage	Cycles	Temperature	Time	Data collection
1	1	95℃	5 min	-
2	50	95℃	10 sec	-
		60℃	30 sec	**FAM /** **CAL Fluor Orange 560** (VIC/HEX/JOE) **/** **Quasar670** (CY5)
		72℃	10 sec	-

11) Run the PCR run immediately.
12) When the PCR operation finished, analyze the data according to the "Results Interpretation" procedures

3. Results Interpretation

Before mutation data analysis, the following items should be checked:

1) Set the threshold to 5% of the maximum fluorescence value (Y axis) of the Positive control (Each fluorescence).
2) For Positive control: Ct value range of each PCs should be 25.0 <Ct < 30.0. If not, the data is *INVALID*. The sample should be retested.
3) For the internal control assay for each sample: Ct values of the samples should be 21.5 <Ct < 40. (Based on copy number of total *EGFR* gene). If Ct value of IC < 21.5, this indicates that there are too many template in reaction. In this case, it would be better to dilute samples and should be retested. If Ct value of IC > 40, this indicates there are insufficient DNA or PCR inhibitors. The sample should be retested with increased or re-extracted DNA.

Analyze the mutation assay for each sample:

1) Record the FAM / CAL Fluor Orange 560 / Quasar 670 Ct values of the samples.

2) Calculate the ΔCt value for each well:

ΔCt value = Sample Ct value (FAM of each MMXs) – Positive control Ct value (FAM of each MMXs)

ΔCt value = Sample Ct value (CFO560 of each MMXs) – Positive control Ct value (CFO560 of each MMXs)

3) Result interpretation for each tube according to the Cut-off ΔCt value in Table 7.

a) If the ΔCt value is< the Cut-off ΔCt value, the sample is determined as positive.
b) If there are no fluorescence signal and Ct values of internal control is 21.5 <Ct < 40, the sample is determined as negative.
c) If the ΔCt value is > the Cut-off ΔCt value, the sample is determined as negative or under the LoD (Limit of Detection) of the kit.
d) Two or more *EGFR* mutations may be detected for a sample.

Table 7-1. Result Determination

EGFR MMXs		Determination
Internal control Ct value	21.5 ≤ Ct ≤ 40	Valid
	Ct < 21.5 or Ct > 40	Invalid
Sample Ct value	Ct < 23.5	Invalid
	Ct > 45 or No signal	Negative

Table 7-2. Cut-off ΔCt value of each targets

Valid result determination		Cut-off ΔCt value	Range	Determination
△Ct Cut-off value	S768I-FAM	10.0	△Ct ≤ 10.0	Positive
			△Ct > 10.0	Negative
	Ex19Del-CFO560	14.5	△Ct ≤ 14.5	Positive
			△Ct > 14.5	Negative
	T790M-FAM	14.5	△Ct ≤ 14.5	Positive
			△Ct > 14.5	Negative
	L861Q-FAM	13.0	△Ct ≤ 13.0	Positive
			△Ct > 13.0	Negative
	G719X-CFO560	8.5	△Ct ≤ 8.5	Positive
			△Ct > 8.5	Negative
	Ex20Ins-FAM	16.0	△Ct ≤ 16.0	Positive
			△Ct > 16.0	Negative
	L858R-CFO560	10.0	△Ct ≤ 10.0	Positive
			△Ct > 10.0	Negative

Performance Characteristics

The performance characteristics of this kit were validated on AB 7500 Fast.

1. Limit of Detection

The Low Limit of Detection (LLoD) of the kit for each mutation is shown in Table 8.

2. Specificity

Structurally related EGFR protein analog sequences (HER2, HER3 and HER4) have been shown not to cross-react with the ADPS™ EGFR Mutation Test kit when the potential cross-reactive sequence was added at a genomic copy number equivalent to 99 ng/PCR input to the extracted DNA prior to the amplification. A control condition without plasmid DNA was included. There were no cross-reactivity with each HER2, HER3 and HER4 plasmids. Additionally, the EGFR exon 19 mutation L747S was tested for cross reactivity. Results indicated that the ADPS™ EGFR Mutation Test kit cross-reacts with the EGFR exon 19 mutation L747S.

3. Interference factor

Triglycerides (37 mM), albumin (60 g/L) and hemoglobin (1.5 g/L) have been shown not to interfere with the ADPS™ EGFR Mutation Test kit when the potentially interfering substance was added to healthy-donor K2 EDTA plasma samples containing wild-type and mutant EGFR sequences.

The study results demonstrate that EDTA, Neupogen, and Erlotinib do not interfere with the performance of the ADPS™ EGFR Mutation Test kit when the potentially interfering substance was added to healthy-donor K2 EDTA plasma samples containing wild-type and mutant EGFR sequences.

4. Precision

The precision strudy of the ADPS™ EGFR Mutation Test kit was assessed using 3 diluted EGFR mutation specific plasmids blends with wild-type genomic DNA samples including: 1%, 0.01% mutant contents. 3 These samples were tested in duplicate by two operators, using three different reagent lots and three AB 7500 Fast over 3 non-consecutive days. The Ct values were calculated, the CV values were all within 5%.

Table 8. LLoD of each EGFR mutations

Exon	Mutation	EGFR Nucleic Acid Sequence	COSMIC ID	LoD (Copies)	Detection Sensitivity (%)
Exon 18	G719X	2155G>A	6252	15	0.05
		2155G>T	6253	10	0.03
		2156G>C	6239	3	0.01
Exon 19	Ex19Del	2240_2251del12	6210	3	0.01
		2239_2247del9	6218	3	0.01
		2238_2255del18	6220	3	0.01
		2235_2249del15	6223	3	0.01
		2236_2250del15	6225	3	0.01
		2239_2253del15	6254	3	0.01
		2239_2256del18	6255	3	0.01
		2237_2254del18	12367	3	0.01
		2240_2254del15	12369	3	0.01
		2240_2257del18	12370	3	0.01
		2239_2248TTAAGAGAAG>C	12382	3	0.01
		2239_2251>C	12383	3	0.01
		2237_2255>T	12384	3	0.01
		2235_2255>AAT	12385	3	0.01
		2237_2252>T	12386	3	0.01
		2239_2258>CA	12387	3	0.01
		2239_2256>CAA	12403	3	0.01
		2237_2253>TTGCT	12416	3	0.01
		2238_2252>GCA	12419	3	0.01
		2238_2248>GC	12422	3	0.01
		2237_2251del15	12678	3	0.01
		2236_2253del18	12728	3	0.01
		2235_2248>AATTC	13550	3	0.01
		2235_2252>AAT	13551	3	0.01
		2235_2251>AATTC	13552	3	0.01
		2253_2276del24	13556	3	0.01
		2237_2257>TCT	18427	3	0.01
		2238_2252del15	23571	3	0.01
		2233_2247del15	26038	3	0.01
		2232_2249del15	221565	3	0.01
		2234_2248del15	1190791	3	0.01
Exon 20	S768I	2303G>T	6241	3	0.01
	T790M	2369C>T	6240	10	0.03
	Ex20Ins	2307_2308ins9GCCAGCGTG	12376	3	0.01
		2319_2320insCAC	12377	3	0.01
		2310_2311insGGT	12378	3	0.01
		2311_2312ins9GCGTGGACA	13428	3	0.01
		2309_2310AC>CCAGCGTGGAT	13558	3	0.01
Exon 21	L858R	2573T>G	6224	3	0.01
		2573_2574TG>GT	12429	3	0.01
	L861Q	2582T>A	6213	3	0.01

Limitations

1) The kit is to be used only by personnel specially trained with PCR techniques.
2) The 0.01 % of detection sensitivity is guaranteed only when input DNA is used with maximum of 30,000 copies.
3) The results should never be used for *in vitro* diagnosis (IVD).
4) The kit has been validated for use with circulating DNA extracted from plasma sample and human genomic DNA extracted from FFPE tissue.
5) Reliable results are dependent on proper specimen collection, processing, transport, and storage.
6) The sample containing degraded DNA may affect the ability of the test to detect *EGFR* mutations.
7) This kit can only assess the *EGFR* mutation status and detect 44 *EGFR* mutations indicated above.
8) Samples with negative result (No mutation detected) may harbor another *EGFR* mutations not detected by this assay.

References

1) Herbst RS. Review of epidermal growth factor receptor biology. Int. J. Radiat. Oncol. Biol. Phys. 2004. 59 (2 Suppl): 21–6.
2) Oda K, Matsuoka Y, Funahashi A, Kitano H. A comprehensive pathway map of epidermal growth factor receptor signaling. Mol. Syst. Biol. 2005. 1:2005.0010.
3) Lynch TJ, Bell DW, Sordella R, et al. 2004. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N. Engl. J. Med. 2004. 350(21):2129-39.
4) Pérez-Soler R, Chachoua A, Hammond LA, et al. Determinants of tumor response and survival with erlotinib in patients with non--small-cell lung cancer. J. Clin. Oncol. 2004. 22 (16): 3238–47.
5) Pao W, Miller VA, Politi KA, et al. Acquired resistance of Lung Adenocarcinomas to Gefitinib or Erlotinib is associates with a second mutation in the EGFR kinase domain. Plos Med. 2005. 2(3):e73.
6) Pao W, Chmielecki J. Rational, biologically based treatment of EGFR-mutant non-small-cell lung cancer. Nat Rev Cancer. 2010;10:760-774.
7) Clinical and Laboratory Standards Institute (CLSI). Protection of laboratory workers from occupationally acquired infections. Approved Guideline-Fourth Edition. CLSI Document M29-A4: Wayne, PA;CLSI, 2014.
8) Clinical and Laboratory Standards Institute (CLSI). Interference testing in clinical chemistry; Approved Guideline–Third Edition. CLSI Document EP07 Appendix A:Wayne, PA; CLSI, 2018.
9) Clinical and Laboratory Standards Institute (CLSI). Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline - Second Edition. CLSI Document EP17-A2: Wayne, PA; CLSI, Jun 2012.

Symbol Information

Symbol	Title of symbol	Symbol	Title of symbol
	Temperature limit		Caution
	Use-by date		Consult instructions for use
LOT	Batch code		
REF	Catalogue number		
	Manufacturer (Name&Address)		
Σ	Contains sufficient for <*n*> tests		