

Instructions for use

eazyplex[®] SuperBug AmpC

**Molecular biological rapid test
for the detection of AmpC β -lactamases**







for use with Genie[®] II Mk2 devices

For in vitro diagnostic use



eazyplex[®] SuperBug AmpC	REF: 7606
language: english	valid from: April 2019

Explanation of symbols

IVD	in vitro diagnostic medical device
LOT	Batch code
REF	Catalogue number
	Use by
	Temperature limitation
TESTSTRIP	Teststrip
	Contains sufficient for <n> tests
	Consult instructions for use
	No re-use
	Manufacturer

Document Revision Information:

Actualization of symbols according to EN ISO 15223-1, page numbers inserted, software version actualised (4.2), „select the test manually“deleted (8.1), Documentation under 9. amended by PDF report and CSV file, Warning messages inserted under 9., 11. Troubleshooting amended, general improvement of phrases.

1. Intended Use

The **eazyplex[®] SuperBug AmpC** test system (Cat. No. 7606) is a qualitative in vitro diagnostic medical device for the detection of different genes, which confer a variety of resistances in gram negative bacteria to β -lactam antibiotics.

The test can be performed at any time by qualified professional staff in a medical laboratory. The intended use includes:

- diagnosis (confirmatory assay to verify results of previous testing) and aid to diagnosis (providing additional information to assist in the determination or verification of a patient's clinical status, test is not the sole determinant) of all kind of patients via testing bacterial colonies.

2. AmpC enzymes

AmpC enzymes differ from ESBL in two substantial functional points. On the one hand, in addition to the oxyimino-Cephalosporins also cephamycins are included in the substrate spectrum. On the other hand, there is no inhibition by clavulanic acid or Tazobactam. Due to these characteristics, classification into group 1 by Bush et al. (1995) resulted. AmpC belong to group C in the Ambler classification scheme.

Many *Enterobacteriaceae* like *Enterobacter cloacae*, *Citrobacter freundii* and *Serratia marcescens* possess chromosomal coded AmpC with inducible expression, which can lead to a resistance against β -lactam antibiotics.

Other species, like *E. coli*, exhibit a constitutively produced chromosomal coded AmpC. Based on mutations in the promotor, hyper-production and therefore resistance may appear in these cases.

Klebsiella spp. and *Proteus mirabilis* are representatives for *Enterobacteriaceae* which don't possess chromosomal located AmpC-genes. The first plasmid encoded AmpC gene was detected in *K. pneumoniae* isolates at the beginning of the 1990s. Due to a similarity of 90% to the chromosomal AmpC gene from *Enterobacter cloacae*, genesis is suspected out of the chromosomal variant. In combination with porin loss, plasmid encoded AmpC can even lead to a resistance against carbapenemes.

In the meantime, there are different plasmid encoded AmpC families worldwide. The family of CMY (cephamycinase) lactamases represents the major group with 139 members, followed by ACT („AmpC type“) lactamases (39 members), DHA (Dharan hospital in Saudi Arabia) lactamases (24 members) und MIR (Miriam hospital in Providence) lactamases (18 members).

3. Principle of the test

A single **eazyplex**[®] test strip contains six oligonucleotide primers in each filled cap and these provide the means for simultaneous, specific amplification of different genes in a single isothermal amplification reaction. In the presence of relevant DNA sequences, specific amplification products are generated and visualised by real-time fluorescence measurement of a fluorescence dye bound to double-stranded DNA. Thus, positive signals indicate the presence of the corresponding genes in the sample to be investigated and a clear genotype determination of a (potential) resistance to different β -lactams due to AmpC- β -lactamases is possible.

Data interpretation is based on an algorithm in the eazyReport[™] software.

The following protective mechanisms prevent the use of false results:

Performance of “inhibition control” with each sample prevents the use of false negative test results due to inhibition of the amplification reaction and simultaneously serves as reagent control.

As required, a test strip can be processed as negative / contamination control by testing **RALF** without addition of sample material. In this case, only the inhibition control is allowed to create a positive signal.

tube-n°	Assay parameter (abbreviation in result display)	Specificity	Colour of curve
1	ACC	ACC-1 to -6	red
2	Inhibition control	Inhibition control	orange
3	CMY-II	CMY-II group	yellow
4	DHA	DHA-1 to -24	light green
5	MOX	MOX-1/2/3/4/8/10/11 CMY-1/8/9/10/11/19	dark green
6-8	--	--	--

4. Reagents

4.1 Content

The reagents contained in one kit are sufficient for 24 determinations.
Each kit contains:

TESTSTRIP	Test strips with 5 filled tubes, each containing lyophilized, ready-to-use mix for isothermal amplification. The mix contains DNA-polymerase, buffer components, Mg ₂ SO ₄ , dNTPs, oligonucleotide primers and a fluorescence dye.	24 strips
RALF	2ml –tubes with 500 µl „RALF - Resuspension and Lysis Fluid“ each (ready for use)	24 x 500 µl

4.2 Additional accessories required

- GENIE® II Mk 2 with eazyReport™ - software version 2.34 or higher (including Instructions for Use in PDF format)
- Inoculation needle
- Heating block for 2 ml tubes
- Pipettes with sterile disposable filter tips
- optional: USB bar code scanner
- optional: printer DYMO® Labelwriter 450 (Dymo) with labels 54x101mm

5. Warnings and precautions

- ☞ All reagents and materials which come into contact with potentially infectious samples must be treated with suitable disinfectant or autoclaved.
- ☞ Suitable disposable gloves must be worn during the entire test.
- ☞ **Never** open a test strip after use! Autoclave used test strips!

6. Handling notes – preparation of assay realization

The components of **eazyplex[®] SuperBug AmpC** have to be stored from 15°C to 30°C.

The kits have an expiry date. Quality cannot be guaranteed after this date.

Before beginning the test, remove a test strip from the bag. The test strip may only be used if the white pellets in the filled tubes are visible.

As with any test procedure, good laboratory practice is essential to the proper performance of this assay.

7. Sample material

The sample material has to be:

-bacterial colonies from agar plates, selective media recommended

Any swabs, blood or stool samples **CANNOT** be used.

8. Test procedure

8.1 Preparation of the system

- Turn on GENIE[®] II Mk2
- Touch the Screen
- Enter user name and password
- Select „Run“
- Scan test barcode via barcode scanner or enter test barcode manually
- Check display if the right test profile is selected
- Enter patient´s / sample ID (via barcode scanner or keyboard)
- Confirm the selection with “Enter”

8.2 Preparing the amplification reaction

Suspend a small part of a single bacterial colony in 500 µl **RALF** by an inoculation needle. As soon as a little amount of cell material is visible on the inoculation needle, it is sufficient sample material for the test.

 **Caution! Too much cell material may considerably reduce the effectiveness of the reaction and lead to invalid test runs.**

Incubate this **RALF** -suspension at 99°C for 2 minutes for cell lysis. If you wish further microbiological testing, transfer 50 µl of the cell suspension in a sterile tube **before** cell lysis.

Carefully remove the protective foil from the test strip.

Pipette 25 µl of the **RALF**-suspension onto the ready-to-use mix in each filled tube of the test strip, taking care not to allow the pipette tip to make contact with the pellet. Do not vortex, shake heavily or pipette up and down. Remove any air bubbles by tapping the test strip gently.

Once the pellets have dissolved, place the test strip immediately into the GENIE® II Mk2 device and start the run (8.3).

8.3 Realization of the amplification reaction

- Select „Start“
- Select block A or B
- Place the test strip into the selected block
- Close the lid
- Start test run by selecting „Yes“.
- If the second block is not in use, a second test run can be initiated by the button “start test run” (see 8.1)
- Note: the directory path and number of test run data file can be seen on the display
- Once the run is complete, open the lid and remove the test strip, taking care as the block could still be hot!
- **Never** open a test strip after use! Danger of contamination!

9. Evaluation

The test run can be monitored in real time mode (choose „Amplification“). Positive results are indicated by a strong rise in fluorescence signal (in the form of a typical amplification curve). Unambiguous assignment of the curves to the test parameters takes place by coloring.

After completion of the test run select „Results“:

Valid test run:

Positive results are colored red.

Invalid test run:

The result of „Inhibition Control“ is colored red (invalid). In this case, data interpretation of the eazyReport™ software is displayed above the result table as follows:

„Invalid control“ (colored red)

Warning messages:

„WARNING! Kit expired!“:

A kit has been used which is out of date (see 6.).

„WARNING! Too much sample material!“:

Too much sample material was used; this could have been the reason for an invalid test run (see 11.)

Documentation:

- Result printout via Dymo® Labelwriter 450 printer: select „print“
or
- create a PDF result report: select „PDF“; the generated PDF file is stored in the folder „Report“ on the device and can be exported via USB stick (according to Instructions for Use Genie® II Mk2).
or
- create a result file in CSV format: select „CSV“; the generated CSV file is stored in the folder „Report“ on the device.

The stored run file can be reviewed at any time:

- select symbol „Folder“
- select „LOG“ and confirm with „✓“
- the data files are numbered consecutively and archived according to creation date

10. Interpretation of the test results

The **eazyplex[®] SuperBug AmpC** system is a rapid confirmatory test for the specific detection of different AmpC gene families in bacterial isolates.

Positive results in the **eazyplex[®] SuperBug AmpC** system demonstrate the presence of the resistance gene in an isolate. This does not mean that the genes are also actually expressed. The pathogen, however, possesses the potential to express the gene. No conclusions on, for example, the minimal inhibiting concentration of β -lactam antibiotics can be made based on the results of this test system.

The **eazyplex[®] SuperBug AmpC** system is neither intended to diagnose an infection with β -lactam resistant bacteria (it cannot be distinguished between colonisation and infection) nor to guide or monitor medical treatment.

The test solely generates a test result. The attending doctor is responsible for achieving a diagnosis and a decision about the treatment of the patient and / or appropriate hygienic measures.

11. Troubleshooting

All signals negative (incl. inhibition control):

- Too much cell material was used for amplification → suspend just a small part of one colony in 500 μ l RALF or dilute already suspended cell material with RALF.
- If assay with diluted sample is invalid again → perform assay with RALF without sample.
- If inhibition control is negative again → device may be damaged, please contact our support team.

All signals positive (incl. inhibition control):

Very occasionally cell lysis in a sample will not complete within the first 3 minutes of the test run (signals are not displayed at the beginning of the run). If cell lysis occurs later on during the amplification reaction, newly appearing dsDNA will lead to unspecific amplification signals. This phenomenon can be identified easily because it will happen simultaneously in all wells (but the IC curve or correct positive amplification curves could appear earlier). → It is recommended to repeat the test run with fresh sample material with an extended incubation time of 10 minutes for complete cell lysis.

→ If the phenomenon reoccurs, the sample should be interpreted as "invalid" or "not valid".

12. Performance data

As part of a retrospective evaluation study at the Robert-Koch-Institut Wernigerode (Dr. Yvonne Pfeiffer), 50 different enterobacterial isolates were tested in comparison to in-house PCR with the following results:

	in-house PCR			
	positive			negative
	ACC	DHA	CMY-II	MOX
eazyplex[®] positive ACC	9	0	1*	0
eazyplex[®] positive DHA	0	8	1*	0
eazyplex[®] positive CMY-II	0	0	24	0
eazyplex[®] positive MOX	0	0	1*	1
eazyplex[®] negative				8

***Remark:**

In a correct positive CMY-II sample ($T_t=6:30$) positive signals for ACC, DHA and MOX appeared ($T_t=14:15$ up to $T_t=15:30$), presumably due to additional cell lysis.

The following analytical performance data result:

Sensitivity (overall AmpC): 100 % (42 of 42 positive samples)
 Specificity (overall): 98 % (155 of 158 negatives)

13. References

1. Ambler, RP. The structure of beta-lactamases. *Philos Trans R Soc Lond B Biol Sci*, 1980. 289(1036):321-31.
2. Bush, K, GA Jacoby, and AA Medeiros. A functional classification scheme for beta-lactamases and its correlation with molecular structure. *Antimicrob Agents Chemother*, 1995. 39 (6):1211-33.
3. Bush, K and Jacoby, GA. Updated functional classification of beta-lactamases. *Antimicrob Agents Chemother*, 2010. 54(3):969-76.
4. Philippon, A, G Arlet, and GA Jacoby. Plasmid-determined AmpC-type beta-lactamases. *Antimicrob Agents Chemother*, 2002. 46(1):1-11.
5. Papanicolaou, GA, AA Medeiros, and GA Jacoby. Novel plasmid-mediated betalactamase (MIR-1) conferring resistance to oxyimino- and alpha-methoxy betalactams in clinical isolates of *Klebsiella pneumoniae*. *Antimicrob Agents Chemother*, 1990. 34(11):2200-9.
6. Jacoby GA, AmpC beta-lactamases. *Clin. Microbiol. Rev.* January 2009 vol. 22 no. 1 161-182
7. Voets GM, Fluit AC, Scharringa J, Schapendonk C, van den Munckhof T, Leverstein-van Hall MA, Stuart JC. Identical plasmid AmpC beta-lactamase genes and plasmid types in *E. coli* isolates from patients and poultry meat in the Netherlands. *Int J Food Microbiol.* 2013 Nov 1;167(3):359-62
8. Seiffert SN, Carattoli A, Tinguely R, Lupo A, Perreten V, Endimiani A. High prevalence of extended-spectrum β -lactamase, plasmid-mediated AmpC, and carbapenemase genes in pet food. *Antimicrob Agents Chemother.* 2014 Oct;58(10):6320-3
9. Bauernfeind A, Stemplinger I, Jungwirth R, Giamarellou H. Characterization of the plasmidic beta-lactamase CMY-2, which is responsible for cephamycin resistance. *Antimicrob Agents Chemother.* 1996 Jan;40(1):221-4.
10. Mata C, Miró E, Toleman M, Rivera MA, Walsh TR, Navarro F. Association of bla(DHA-1) and qnrB genes carried by broad-host-range plasmids among isolates of *Enterobacteriaceae* at a Spanish hospital. *Clin Microbiol Infect.* 2011 Oct;17(10)
11. Roh KH, Song W, Chung HS, Lee YS, Yum JH, Yi HN, Chun JS, Yong D, Lee K, Chong Y. Chromosomal cephalosporinase in *Enterobacter hormaechei* as an ancestor of ACT-1 plasmid-mediated AmpC β -lactamase. *J Med Microbiol.* 2012 Jan;61(Pt 1):94-100.
12. Raskine L, Borrel I, Barnaud G, Boyer S, Hanau-Berçot B, Gravisse J, Labia R, Arlet G, Sanson-Le-Pors MJ. Novel plasmid-encoded class C beta-lactamase (MOX-2) in *Klebsiella pneumoniae* from Greece. *Antimicrob Agents Chemother.* 2002 Jul;46(7):2262-5.
13. <ftp://ftp.ncbi.nlm.nih.gov/pathogen/betalactamases/Allele.tab> (Nov 2016).

The purchase price of this product includes a non-transferable license under European Patents EP1020534, EP1873260, EP2045337, EP2287338, EP1275715, EP2253717, EP1327679, EP2031058 or their foreign counterparts, owned by Eiken Chemical Co., Ltd.



AmplexDiagnostics GmbH
Werkstraße 2
83555 Gars-Bahnhof
Germany

Support:
Tel.: +49 (0) 8073 / 9 16 93-50
Fax: +49 (0) 8073 / 9 16 93-55
support@eazyplex.com