Read this Package Insert carefully before testing.

For in vitro diagnostic use only

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Approval number 30300EZX00099000

MIZUHO MEDY Co., Ltd.

Helicobacter pylori detection kit

Smart Gene® H. pylori G
Test cartridge

[General precautions]
1) This product is for in vitro diagnostic use only. Do not use it for other purposes.
2) A diagnosis of *Helicobacter pylori* infection and clarithromycin-resistance should be made not only based on the test results of this product but also comprehensive judgment from the assessment of clinical symptoms.
3) Procedures not described in Package Insert are not guaranteed.
4) Do not disassemble the test cartridge of this product under any circumstances.
5) If extraction reagent solution contacts the skin, eye, or mouth, flush with plenty of water as first aid, and see a doctor if necessary.
6) Before using the dedicated instrument "Fully automated gene analysis instrument Smart Gene®", be sure to read its Package Insert and the User Manual thoroughly.

[Contents]
1) Test cartridge
   - KOD exo(-)DNA polymerase
   - Deoxyadenosine triphosphate (dATP)
   - Deoxythymidine triphosphate (dTTP)
   - Deoxyguanosine triphosphate (dGTP)
   - Deoxycytidine triphosphate (dCTP)
   - *Helicobacter pylori* specific forward primer
   - *Helicobacter pylori* specific reverse primer
   - *Helicobacter pylori* specific QProbe
2) Extraction reagent solution (specimen collection set, sold separately)
   The extraction reagent solution is a buffer solution containing detergents and chaotropic salts.

[Intended use]
For detection of *Helicobacter pylori* DNA and mutations in domain V of the 23S rRNA in the intragastric fluid obtained during endoscopic examination. (An aid in the diagnosis of *Helicobacter pylori* infection and clarithromycin-resistance)

[Principle of the test]
The Smart Gene H. pylori G is a reagent for detection of *Helicobacter pylori* DNA and mutations relating clarithromycin susceptibility in domain V of the 23S rRNA by the RT-PCR (Reverse Transcription Polymerase Chain Reaction) using a fluorescent labeling probe (QProbe)).
The test cartridge contains KOD exo(-) DNA polymerase, substrates (Deoxyadenosine triphosphate, Deoxythymidine triphosphate, Deoxyguanosine triphosphate, Deoxycytidine triphosphate), primer set binding to 23S rRNA gene region of Helicobacter pylori DNA (*Helicobacter pylori* specific forward primer, *Helicobacter pylori* specific reverse primer), and *Helicobacter pylori* specific QProbe binding to 23S rRNA gene region including positions of 2142 and 2143 relating clarithromycin susceptibility.
When a sample is added in the sample spot of the test cartridge and inserted into the dedicated instrument, if the target gene of *Helicobacter pylori* DNA is present in the sample, the DNA is absorbed to silica particle which is on the membrane filter and is washed with the buffer in the test cartridge. The membrane filter is then inserted into the reaction tube, and the reverse transcription and nucleic acid amplification will be performed according to the temperature profile. Because the specific QProbe binding to the target gene results in fluorescence quenching, the quenching caused by the interaction between specific QProbe and the amplified product of target DNA is detected and determined by a dedicated instrument.
In addition, to confirm that the operation of measurement and nucleic acid amplification has proceeded properly, the reagent contains DNA internal standard and internal standard of QProbe. If quenching of the specific QProbe is not detected, the quenching by amplified products of the DNA internal standard is detected and determined negative.

[Procedural precautions]
1) This product is dedicated to being used with "Fully automated gene analysis instrument Smart Gene®".
2) Do not use the specimen which is extremely cloudy due to the food residue.
3) If there is a sediment in extraction reagent solution, warm the solution to dissolve the sediment before use.
4) Collected specimen should be prepared as a sample in accordance with after-mentioned "Sample preparation" in [Test procedure] and tested as soon as possible. If specimens cannot be tested immediately, specimens extracted in the extraction reagent solution can be held at 2 to 8 ℃ for up to 7 days. When storing stated still as specimen(intragastric fluid), which can be held at 2 to 30 ℃ for up to 3 days. Please note that the gene could be broken during the storage in case the acid level of specimen is high.
5) When adding the sample, make each droplet carefully, while avoiding the tip of the filter to contact with the test cartridge, and add the predetermined volume (4 drops) to the sample spot. If the volume of added sample is excessive or insufficient, correct test results may not be obtained.
6) Hold the holding part on the side of the test cartridge and avoid touching the sample spot and the reaction tube by hand.
7) Do not give a strong impact on the test cartridge such as dropping.
8) Interfering substances and medications
   Following substances and blood at the concentration listed below did not interfere with the performance of this product:

Amoxicillin (100μg/mL)	Clarithromycin (100μg/mL)
Metronidazole (100μg/mL)	Levofloxacin (100μg/mL)
Sitafloxacin (100μg/mL)	Lansoprazole (100μg/mL)
Vonoprazan (100μg/mL)	Famotidine (100μg/mL)
Dimeticone (100μg/mL)	Indigocarmine (100μg/mL)
Compound iodine glycerin (1%)	Sodium bicarbonate (100μg/mL)
Pronase (100unit/mL)	Lidocaine (100μg/mL)
Naphazoline nitrate (100μg/mL)	Mint oil (1%)
Indirect Bilirubin (20mg/dL)	Conjugated bilirubin (20mg/dL)
Hemoglobin (500mg/dL)	Chyle (2000 Formazin Turbidity Unit)
Human genome (28,000 copies/mL)	Blood (0.5%)

9) Cross reactivity

Cross reactivity with the following virus and bacteria were not observed.

• Bacteria

Bacteroides culgatus	*Bifidobacterium adolescentis*
Bifidobacterium breve	*Bifidobacterium infantis*
Campylobacter jejuni	*Candida albicans*
Citrobacter freundii	*Clostridioides difficile*
Clostridium perfringes	*Enterobacter cloacae*
Enterococcus faecalis	*Escherichia coli*
Klebsiella pneumoniae	*Lactobacillus gasseri*
Lactobacillus Lactis	*Lactobacillus reuteri*
Listeria monocytogenes	*Neisseria meningitidis*
Proteus mirabilis	*Pseudomonas aeruginosa*
Salmonella enteritidis	*Staphylococcus aureus*
Staphylococcus epidermidis	*Streptococcus agalactiae*
Streptococcus pyogene	

Regarding bacteria belonging to *Helicobacter* (11 species) below, the cross reactivity was confirmed by creating artificial gene synthesis of the sequence where the primer prove of this product is bind confirming each sequence through NCBI database. As a result, it was confirmed that cross reactivity with the concentration shown as the table below were not observed.

Bacteria belonging to *Helicobacter* (artificial gene synthesis)	Strain	Concentration
H. bizzozeronii	CCUG 35545	10^7 copies/μL
H. felis	ATCC 49179	10^6 copies/μL
	Lee CS3	10^2 copies/μL
H. suis	HS1	10^7 copies/μL
H. helimonnii	ASB6	10^3 copies/μL
	ASB1.4	10^1 copies/μL
H. salomonis	Isolate 56878_4	10^6 copies/μL
H. bilis	ATCC 49314	10^7 copies/μL
H. canadensis	ATCC 700969	10^7 copies/μL
H. pullorum	NCTC 12827	10^7 copies/μL
H. canis	ATCC 51402	10^7 copies/μL
H. cinaedi	CCUG 18818	10^7 copies/μL
H. fenneliae	CCUG 18820	10^7 copies/μL

According to the table above, cross reactivity with *H. bizzozeronii, H. suis, H. -salomonis, H. bilis, H. canadensis, H. pullorum, H. canis, H. cinaedi, H.-fenneliae* does not seem to be observed. *H. helimannii* may be cross reacted. *H.-felis* may also be cross reacted depending on the strain.

10) Contamination prevention

Contamination of trace amounts of the target gene and amplified product may cause false positives because this product uses the PCR method as the measurement principle. Please note that false positive results may occur in an environment contaminated by using other PCR equipment together. Since PCR by this product is carried out in the reaction tube, contamination by the amplified DNA will be prevented. On the other hand, contamination when collecting samples and contamination among specimens cannot be prevented so perform tests according to following instructions.

1. Use the endoscope that has been properly cleaned and disinfected by the washing and disinfection apparatus dedicated for endoscope for the intragastric fluid collection.
2. Wear protective equipment (such as gloves), and change them if the sample adheres.
3. Do not splatter the sample when adding it to the cartridge. When testing multiple specimens, do not remove all test cartridges from aluminum pouches at once. Instead, please open a new pouch after each test cartridge is added with the sample.
4. After adding a sample, put on the filter cap included in specimen collection set on the tip of filter firmly.
5. Dispose of the test cartridge promptly in accordance with the after-mentioned “Precautions for waste disposal” after the test.

[Test procedure]

• Specimen collection

1) Preparation of specimen collection

1. Intragastric fluid collection kit:
   Use Intragastric fluid collection kit (sold separately).
2. Swab:
   Use Nipro sponge swab TYPE L included in specimen collection set (sold separately).
3. Extraction reagent solution:
   Use extraction reagent solution, included in specimen collection set (sold separately) without preparation.

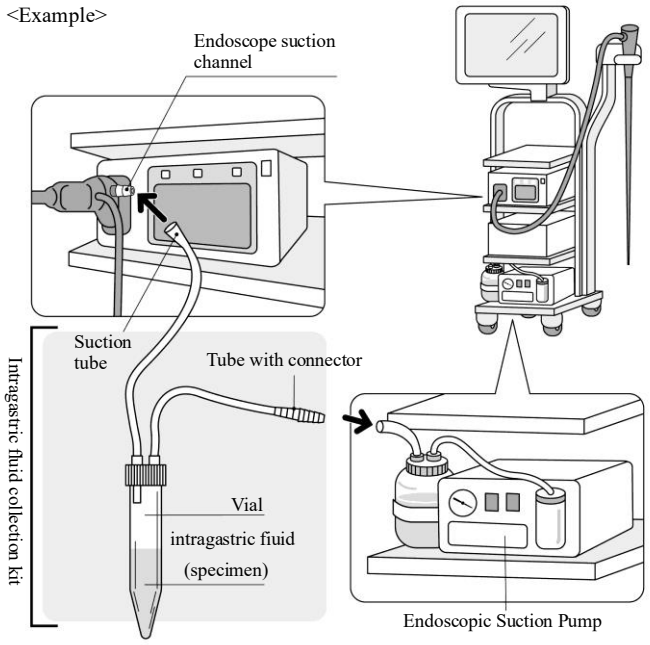
2) Specimen collection

Intragastric fluid:

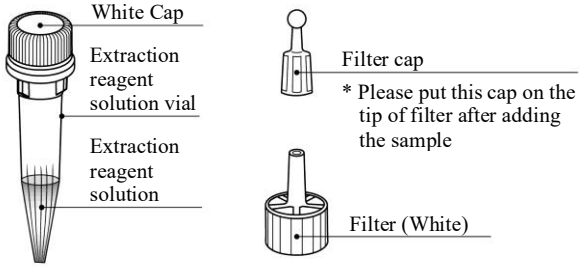
Connect the suction tube of Intragastric fluid Collection kit to suction channel of endoscope and vacuum up intragastric fluid with endoscope. Please use a cap included in this kit when transport the collected specimen. Intragastric fluid for specimen includes intragastric fluid, gas relief medicine (fluid including Pronase, Dimethicon etc.), and the supplied cleansing fluid for washing the gastric mucosa.

Please note that the sensitivity may decrease due to the specimen being diluted when the volume of lavage fluid is large.

<Example>

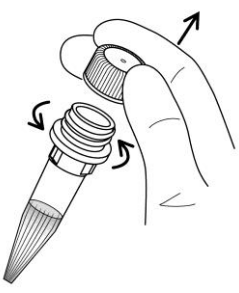


· Detail of extraction reagent solution vial

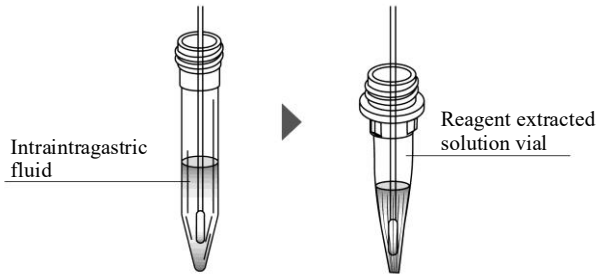


· Sample preparation

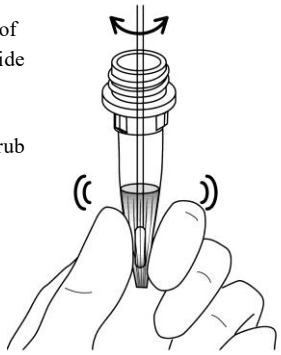
1. Remove the white cap by turning it counterclockwise.



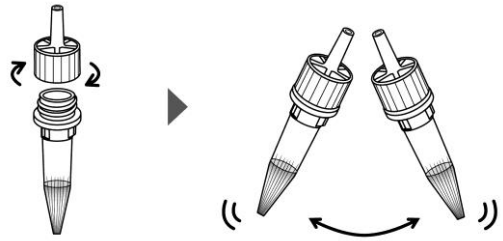
2. Dip the spherical tip into the collected intragastric fluid in the vial of the collection kit with the whole tip soak and leave it for about 10 seconds to collect the specimen. Insert the spherical tip with the specimen into the bottom of the extraction reagent solution vial



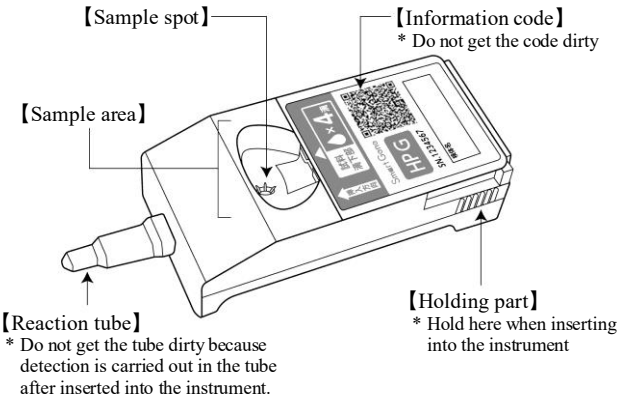
3. Press the spherical tip from the outside of the vial so that it lightly touches the inside wall.
Turn the swab clockwise and counterclockwise about five times and rub the spherical tip on the inside wall and bottom of the vial.
Take out the swab by squeezing out the liquid while pressing the spherical tip against the side of the vial.



4. Install filter firmly, and gently shake the vial several times to mix specimen thoroughly. The sample is ready for use.
* Filter included in the Extraction reagent solution set must be used.



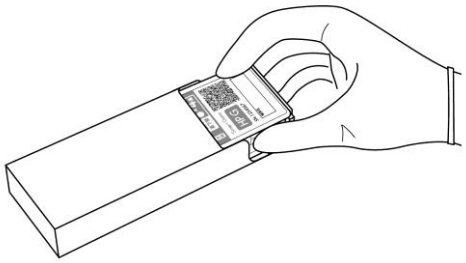
· Details of the test cartridge



· Operation method

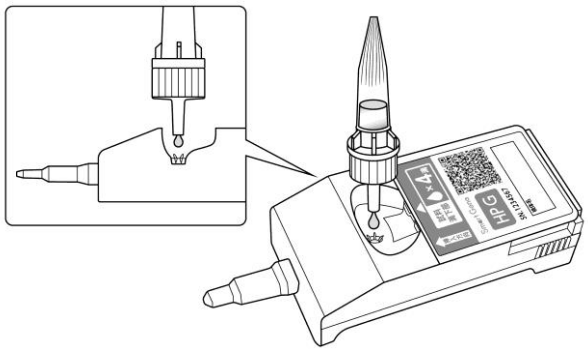
1) Prepare instrument in accordance with the User Manual of the dedicated "Fully automated gene analysis instrument Smart Gene®".
2) Reagent preparation
Use the test cartridge without preparation.
3) Measurement procedure
1. Remove the protective cover containing the test cartridge from the aluminum foil pouch.

2. Pull out the test cartridge from the protective cover.

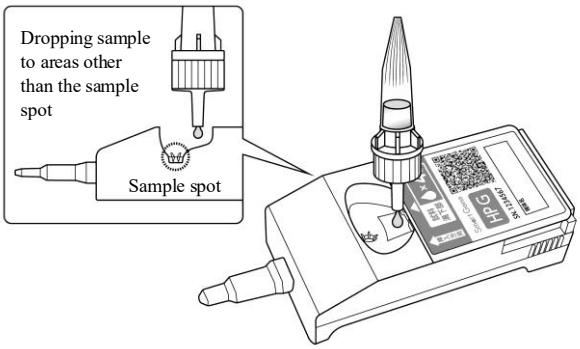


3. Add 4 drops of the sample (approximately 110μL) into the sample spot slowly and precisely from the extraction reagent solution vial which contains the sample.
* Keep the tip of the filter in the sample area and slowly add sample because splatter of the sample or adding sample to areas other than the sample spot may cause a decrease in sensitivity or contamination.

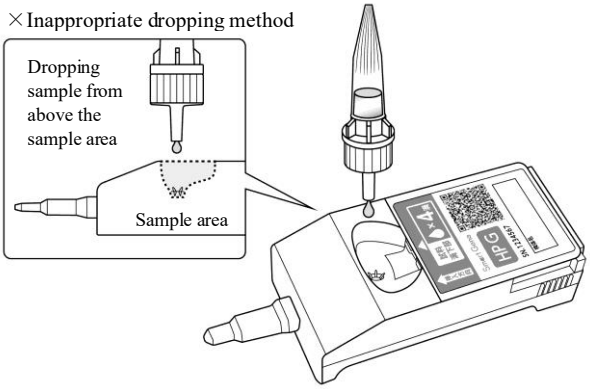
○Adequate dropping method



×Inappropriate dropping method



×Inappropriate dropping method



4. After the sample is added and is absorbed, promptly insert the test cartridge into the insertion slot of the instrument to start the test.

5. Test result is interpreted automatically by the device (in approximately 50 minutes) after the test started.
   ※temperature profile
   (1) 95℃ (98℃ at the first cycle) 10 seconds
   (2) 66℃ or 55℃ 20 seconds
   Repeat (1) and (2) for 50 cycles.
   The target gene is measured at 55℃ and 66℃ (center wavelength 525nm).

[Interpretation]

1) Positive (Mutation -)
   When the amplification of *Helicobacter pylori* DNA is detected and the mutation is not detected by the dedicated instrument, it is interpreted as positive (Mutation -).
2) Positive (Mutation +)
   When the amplification of *Helicobacter pylori* DNA is detected and the mutation is detected by the dedicated instrument, it is interpreted as positive (Mutation +).
3) Negative
   When the amplification of *Helicobacter pylori* DNA is not detected by the dedicated instrument, but the amplification of internal standard DNA is confirmed, it is interpreted as negative.
4) Retest
   When amplification of neither *Helicobacter pylori* DNA nor the internal standard DNA is detected by the dedicated instrument, an operational error can be considered. Check the operation method again and retest with a new test cartridge. If the same result comes out in the retest, use other methods.

・Interpretational precautions

This product is used as an aid in the diagnosis of infection of *Helicobacter pylori* and clarithromycin-resistance. A negative result may be determined if *Helicobacter pylori* DNA in the specimen is below the detection limit of this product, the specimen is inadequately collected, the volume of the specimen is low, the specimen contains a high concentration of interfering substances, etc. The mutation related to the clarithromycin susceptibility is interpreted according to the mutation of the position of 2142 and 2143 on the 23S rRNA gene, hence, other mutations cannot be interpreted. In case the patient is infected with both *Helicobacter pylori* clarithromycin-resistance and *Helicobacter pylori* clarithromycin susceptibility and clarithromycin susceptibility takes up at a higher rate than the clarithromycin-resistance, the mutation may not be detected and may be interpreted as there is no mutation. Make a final definitive diagnosis using comprehensive judgment from the assessment of clinical symptoms and other test results etc.

[Clinical significance]

Helicobacter pylori (H. pylori) infect the intragastric mucus and cause the gastric inflammation. Accompanied by the chronic inflammation of the gastric mucosa, it is also known to cause various upper gastrointestinal complications such as atrophic gastritis, gastric and duodenal ulcers, gastric cancer, and gastric MALT lymphoma[1]. The importance of *H. pylori* eradication therapy as primary prevention of gastric cancer is recognized, as the International Agency of Research on Cancer (IARC), which is the specialized cancer agency of the World Health Organization (WHO), has recommended that appropriate policies to prevent gastric cancer, including *H. pylori* eradication therapy, should be designed in all countries around the world [2].

However, the increase of strains with reduced susceptibility to clarithromycin, one of the drugs used in standard primary eradication in Japan, has become a problem in the eradication therapy of *H. pylori* infection. In selecting drugs for eradication therapy, it is recommended that drug susceptibility testing be performed and that the combination with the highest eradication rate be used.

To diagnose H. pylori infection, the rapid urease test, microscopy, and culture methods, which require endoscopic biopsy tissue, and the urea breath test, anti-H. pylori antibody test, and stool antigen test, which do not require endoscopic biopsy tissue, are used. Resistance to clarithromycin can be determined by performing antibiotic susceptibility test using a culture method, but it takes several days to obtain the result.

This product uses “Intragastric fluid” as a specimen obtained non-invasively during endoscopy. It is a reagent that can detect *H. pylori* DNA and the mutation in the 23 S rRNA gene Domain V region relating to clarithromycin susceptibility by a simple test procedure in a short time.

The clinical performance of the product was evaluated at three medical institutes by collecting intragastric fluid from gastric endoscopic examinations in patients undergoing endoscopy. For the detection of *H. pylori*, the relationship between this product and conventional diagnostic methods, urea breath test and stool antigen test, was evaluated. The discrepancy cases were determined with the results of the Real time PCR method using the intragastric fluid. Regarding the detection of the mutation in the 23S rRNA gene Domain V region relating to clarithromycin susceptibility, the concordance between antibiotic susceptibility test and sequence analysis (Sanger sequencing) was evaluated, and the discrepancy cases were determined with the results by quantitatively analyzed mutation rates via pyrosequencing.

1) *Helicobacter pylori* DNA detection

Comparison with urea breath test

Smart Gene H. pylori

Urea breath test	Positive	Negative	Total
Positive	58	5*2	63
Negative	3*1	98	101
Total	61	103	164

Sensitivity : 92.1% (58/63)
Specificity : 97.0% (98/101)
Accuracy : 95.1% (156/164)

*1 Regarding 3 cases where the results were negative with urea breath test and positive with this product, they were positive with real time PCR.
*2 Regarding 5 cases where the results were positive with urea breath test and negative with this product, they were negative with real time PCR.

Comparison with stool antigen test

Smart Gene H. pylori

Stool antigen test	Positive	Negative	Total
Positive	55	1*4	56
Negative	5*3	96	101
Total	60	97	157

Sensitivity : 98.2% (55/56)
Specificity : 95.0% (96/101)
Accuracy : 96.2% (151/157)

*3 Regarding 4 cases of 5 cases where the results were negative with stool antigen test and positive with this product, they were positive with real time PCR.
*4 Regarding 1 case where the result was positive with stool antigen test and negative with this product, it was negative with real time PCR.

2) The detection of mutations in 23S rRNA gene Domain V region
(The detection of Clarithromycin-Resistant *Helicobacter pylori*)

Comparison with antimicrobial susceptibility test

Smart Gene H. pylori

Antimicrobial susceptibility test	Mutation+	Mutation -	Total
Resistant	18	2*5	20
Susceptible	0	39	39
Total	18	41	59

Total agreement rate: 96.6% (57/59)

*5 Regarding 2 cases where the results were "resistant" with antimicrobial susceptibility test and "Mutation –" with this product, they were quantitatively analyzed by pyrosequencing, and the presence of mutation was 13% and 23% respectively.

Comparison with Sanger sequencing

Smart Gene H. pylori

Sanger sequencing	Mutation+	Mutation -	Total
Resistant	15	0	15
Mixed	3	6*6	9
Susceptible	0	37	37
Total	18	43	61

Total agreement rate: 90.2% (55/61)

*6 Regarding 6 cases where the results were "Mixed" with Sanger sequencing and "Mutation –" with this product, they were quantitatively analyzed by pyrosequencing, and the presence of mutation was 7%, 11%, 13%, 16%, 21%, and 23% respectively.

[Performance characteristics]

1) Performance
   1. Sensitivity
      - When in-house positive control 1 was tested, a positive result (Mutation -) was shown.
      - When in-house positive control 2 was tested, a positive result (Mutation +) was shown.
   2. Accuracy
      - When in-house positive control 1 was tested, a positive result (Mutation -) was shown.
      - When in-house positive control 2 was tested, a positive result (Mutation +) was shown.
      - When in-house negative control was tested, a negative result was shown.
   3. Reproducibility
      - When in-house positive control 1 was tested three times simultaneously, positive results (Mutation -) were shown in all cases.
      - When in-house positive control 2 was tested three times simultaneously, positive results (Mutation +) were shown in all cases.
      - When in-house negative control was tested three times simultaneously, negative results were shown in all cases.
   4. Detection limit
      10 copies/μL

2) Calibration reference material (Standard material)
   Plasmid DNA containing the part of 23S rRNA gene region of *Helicobacter pylori* DNA

[Precautions for use and handling]

1) Precautions for handling (prevention of danger)
   1. Sample (specimen) may contain infectious materials such as HIV, HBV, HCV, etc. Handle sample (specimen) with great care as there is a risk of infection during the test.
   2. Intragastric fluid (specimen) can be highly acidic. At the time of use, wear protective equipment (glasses, disposable gloves, mask, etc.) to avoid sample (specimen) or extraction reagent solution to directly contact skin or eye.
   3. Do not collect the specimen with a swab soaked in the extraction reagent solution.
   4. If a sample (specimen) or extraction reagent solution accidentally gets into your eyes or mouth, take first-aid measures such as rinsing it thoroughly with water, and seek medical attention if necessary.
   5. Do not use the filter cap included in the specimen collection set (sold separately) for transportation or preservation.
   6. Perform the specimen collection under the guidance of a qualified person.
   7. If the sample (specimen) spatters, wipe off with alcohol for disinfection, etc.
2) Precautions for use
   1. Do not freeze the reagent. Store this product in accordance with the description of Package Insert. Do not use frozen reagents as they may change the quality and cause false results.
   2. Do not use this product beyond the expiration date.
   3. Do not store extraction reagent solution vial sideways or upside down.
   4. Use the reagent included in the specimen collection set (sold separately). Do not use other reagents.
   5. Use the test cartridge immediately after opening. If the test cartridge is left in a room for a long time, it may not be measured normally.
   6. Do not touch the sample spot, sample area, and the reaction tube of the test cartridge directly by hand.
   7. Do not put stickers and labels on the test cartridge, as it may cause the failure of the dedicated instrument.
   8. Handle the test cartridge carefully as scratches or dust on the reaction tube may influence the measurement.
   9. Handle the test cartridge carefully as scratches or dust on the information code may influence the measurement.
   10. Do not use the reagent of this product except for the purpose of this testing.
   11. Test cartridge, swab, and extraction reagent solution vial (including filter and cap) are intended for single use only. Do not disassemble the test cartridge after using it.
   12. Use Nipro Sponge Swab TYPE L included in the specimen collection set (sold separately).
   13. Do not touch the spherical tip of the swab before use.
   14. Use swab immediately after opening.
   15. Do not use a swab if a break and/or hole are found on the packaging.
   16. Do not use a swab if it is stained, broken, or bent.
   17. Do not bend or curve the rod of the swab before collecting the specimen.
   18. After preparing the sample, be careful not to spatter the sample when removing the swab.
   19. When preparing samples, extract the specimen by rubbing the spherical tip of the swab on the uneven surface of the inner wall of vails. At that time, please be aware that the spherical tip will break and detach, possibly hindering the addition of the sample if you hold down the spherical tip too strongly from the outside of the vial.
   20. If the amount of specimen collected is excessive or the specimen is highly viscous, the filter may become clogged and adequate sample volume may not be dropped. In that case, collect a new specimen and perform the retest.
   21. Use the filter included in the specimen collection set (sold separately). Using other filters may clog the test cartridge and cause incorrect results.
   22. Liquid (washing buffer) may remain in the sample spot after the test, but it does not affect the testing.
3) Precautions for waste disposal
   1. Sample (specimen) may contain infectious materials such as HIV, HBV, HCV, etc. All samples (specimens), test cartridges, and swabs used in the test must be treated as infectious waste according to the regulations in the facility.
   2. Samples (specimens), used cartridges, and swabs used in testing should be disposed of in a sealed state to prevent contamination upon disposal.
   3. Regarding disposal of used reagents and utensils, dispose of them in accordance with Local Regulation and Law of waste disposal.

[Storage·Expiry]

• Storage : 2 – 8 ℃
  Storage for each constituent reagent
    Test cartridge : 2 – 8 ℃
    Extraction reagent solution (sold separately) : 2 – 30 ℃
• Expiry: 13 months (As indicated on the package)

[Packaging unit]

Smart Gene® H. pylori G Test cartridge 5 tests
  Test cartridge……5 tests

(Sold separately)
Smart Gene® H. pylori G Specimen collection set 10 tests
• Extraction reagent solution vial …… 0.55mL×10 vials
• Accessories
  Nipro Sponge Swab TYPE L (Notification number: 27B1X00045000092)
  …… 10 pieces
  Filter (for extraction reagent solution vial)…… 10 pieces
  Filter cap …… 10 pieces
  Name label…… 1 sheet

(Sold separately)
Intragastric fluid collection kit

[Reference]

1) The Japanese Society for Helicobacter Research Guidelines Committee: Guideline for the Diagnosis and Treatment of H. pylori Infection, Revision Ver. in 2016
2) IARC Working Group Report 2014 Volume 8, Helicobacter pylori eradication as a strategy for preventing gastric cancer
3) Shinya Kurata et al. : Nucleic Acids Res., 29(6), e34(2001)

Technical information
Mizuho Medy Co., Ltd.
Toll-free number (domestic call only)
0120-12-4636
(Hours : MON-FRI (exclude holidays) 9AM-12PM 1PM-5PM)

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https://www.mizuho-m.co.jp/en