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Roche

Diastase Kit

REF 860-004

05279208001

IVD Σ 75

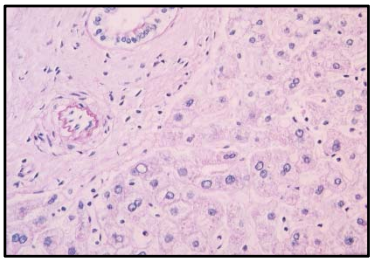

Figure 1. Diastase Kit staining liver tissue.

INTENDED USE

The Diastase Kit, in conjunction with the PAS Staining Kit, is intended for laboratory use as a digestion reagent for glycogen in sections of formalin-fixed, paraffin-embedded (FFPE) tissue stained on a BenchMark Special Stains instrument.

This product should be interpreted by a qualified pathologist in conjunction with histological examination, relevant clinical information, and proper controls.

This product is intended for in vitro diagnostic (IVD) use.

SUMMARY AND EXPLANATION

Diastase Kit is a single bottle kit used in conjunction with the PAS Staining Kit. The PAS Staining Kit is a modification of a technique originally described by McManus in 1946 to visualize mucins, glycogen, basement membrane and fungal organisms through the combination of oxidation of polysaccharides by periodic acid and staining with the Schiff's reagent. [1]

In practice, two sections of the same tissue sample are stained with the PAS Staining Kit: one of them is treated with Diastase in advance, which results in two PAS-stained sections, one with and one without glycogen, that are then compared side-by-side to determine the presence and distribution of glycogen in the sample.

PRINCIPLE OF THE PROCEDURE

Diastase digests the glycogen present in the tissue so it is washed out of the tissue before PAS staining occurs. Diastase digestion is used to differentially determine if the PAS positive component is glycogen. The PAS Staining Kit uses Periodic Acid reagent to oxidize glycols to aldehydes. The Schiff's Reagent reacts with aldehydes to form a colorless dialdehyde compound that is transformed to the magenta staining of glycol containing cellular components.[2]

This kit is optimized for use on BenchMark Special Stains instruments. The reagents are applied to tissue on microscope slides and mixed over the entire specimen.

MATERIAL PROVIDED

The reagent vials are supplied in barcode labeled carriers to insert into the reagent tray of the instrument. Each kit contains sufficient reagent for 75 tests:

One 22 mL vial of Diastase contains approximately 1.2 - 5% diastase of malt, with sodium azide as a preservative.

One vial insert with sipping straw.

Reconstitution, Mixing, Dilution, Titration

No reconstitution, mixing, dilution, or titration of kit reagent is required. Further dilution of the reagent may result in unsatisfactory staining.

MATERIALS REQUIRED BUT NOT PROVIDED

Not all products listed in the method sheet may be available in all geographies. Consult your local support representative.

The following reagents and materials may be required for staining but are not provided:

1. Recommended control tissue
2. Microscope slides, positively charged
3. BenchMark Special Stains instrument
4. BenchMark Special Stains Deparaffinization Solution (10X) (Cat. No. 860-036 / 06523102001)
5. BenchMark Special Stains Liquid Coverslip (Cat. No. 860-034 / 06523072001)
6. BenchMark Special Stains Wash II (Cat. No. 860-041 / 08309817001)
7. PAS Staining Kit (Cat. No. 860-014 / 05279291001)
8. General purpose laboratory equipment

STORAGE AND STABILITY

The Diastase Kit should be stored at 2-8°C. Refrigerated kit components should be brought to room temperature prior to use.

When properly stored, unopened and opened reagent is stable to the date indicated on the label. Do not use reagent beyond the expiration date on the kit.

There are no obvious signs to indicate instability of the reagent; therefore, controls should be run simultaneously with unknown specimens. Contact your local support representative if positive control material shows a decrease in staining as it could indicate reagent instability.

SPECIMEN PREPARATION

Routinely processed, formalin-fixed, paraffin embedded (FFPE) tissues are required for use with this assay and BenchMark Special Stains instruments. The recommended tissue fixative is 10% neutral buffered formalin.[2]

Perform specimen collection and storage according to CLSI document M29-T2.[3] Cut sections to the appropriate thickness, approximately 4 µm, and place the sections on positively charged glass slides.

1. Dry the slides.[2]
2. Print appropriate barcode label(s).
3. Apply barcode labels to the frosted end of the slides prior to loading the slides onto the instrument (see the instrument User Guide for correct application of labels).

Refer to the Instructions for Use section for the recommended protocol for the BenchMark Special Stains instrument.

WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic (IVD) use.
2. For professional use only.
3. CAUTION: In the United States, Federal law restricts this device to sale by or on the order of a physician. (Rx Only)
4. Do not use beyond the specified number of tests.
5. Positively charged slides may be susceptible to environmental stresses resulting in inappropriate staining. Ask your Roche representative for more information on how to use these types of slides.
6. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions. In the event of exposure, the health directives of the responsible authorities should be followed.[4,5]
7. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
8. Avoid microbial contamination of reagents as it may cause incorrect results.
9. This product contains sodium azide (NaN3). Build-up of NaN3 may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with copious amounts of water to prevent azide accumulation in plumbing.
10. Consult local and/or state authorities with regard to recommended method of disposal.
11. For further information on the use of this device, refer to the BenchMark Special Stains instrument User Guide, and instructions for use of all necessary components located at dialog.roche.com.
12. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
13. To report suspected serious incidents related to this device, contact the local Roche representative and the competent authority of the Member State or Country in which the user is established.

This product contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Table 1. Hazard information.

Hazard	Code	Statement
Danger	H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.

Hazard	Code	Statement
	P261	Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
	P284	Wear respiratory protection.
	P304 + P340	IF INHALED: Remove person to fresh air and keep comfortable for breathing.
	P342 + P311	If experiencing respiratory symptoms: Call a POISON CENTER/ doctor.
	P501	Dispose of contents/ container to an approved waste disposal plant.

INSTRUCTIONS FOR USE

Prepare Reagent Vial

Before first use, a vial insert and sipping straw must be placed in the reagent vial.

Remove the shipping cap from the vial and place the insert and straw into the vial. The insert and sipping straw should be left in the vial, once the vial has been opened.

Staining Procedure

1. Load reagents and slides onto the instrument.
2. Place the soft cap into the slot on the reagent holder when the reagent is in use.
3. Perform the staining run according to the recommended protocol in Table 2, and the instructions in the User Guide.
4. When the run is complete, remove the slides from the instrument.
5. Use the soft cap to cover the reagent vial when reagent is not in use.
6. After use, store the reagents according to the recommended storage conditions.

Recommended Protocol

The parameters for the automated procedures can be displayed, printed and edited according to the procedure in the instrument User Guide.

The following procedures allow flexibility to accommodate user preference. This product has been optimized for use with the BenchMark Special Stains instrument but the user must validate results obtained with this product.

Table 2. Recommended staining protocol for Diastase Kit in conjunction with PAS Staining Kit on a BenchMark Special Stains instrument.

Staining Procedure	S PAS
Protocol Step	Method
Deparaffinization	Select to automate paraffin removal.
Baking (optional)	The default is not selected. 75°C for 4 minutes is recommended
Diastase	Select to enable Diastase and options for PAS Diastase.
Optimize Diastase (PAS Diastase)	The default time is 4 minutes. Select to enable adjustment of incubation time from 4 to 16 minutes.
PAS Alcian Blue for Diastase* (optional)	Not selected. This option must be validated by the user.
Optimize PAS AB Intensity	The default time is 8 minutes. Select an incubation time from 8 to 16 minutes:** 8 minutes, lighter staining of acid mucins 16 minutes, darker staining of acid mucins
Optimize Schiff's for PAS D (PAS Schiff's)	The default is 50°C for 20 minutes.
Optimize Hematoxylin (PAS Hematoxylin)	The default time is 8 minutes. Select to enable adjustment of incubation time:** 4 minutes, lighter nuclear staining 12 minutes, darker nuclear staining
	Select to enable adjustment of staining intensity:** Select a temperature from 37-60°C: 37°C, lighter Schiff's staining intensity 60°C, darker Schiff's staining intensity Select an incubation time from 12 to 20 minutes: 12 minutes, lighter Schiff's staining intensity 20 minutes, darker Schiff's staining intensity

* Additional procedure options are available for products that can be used in conjunction with the PAS Staining Kit and Diastase Kit.

** To adjust staining preferences, increment the stain temperature and incubation time one parameter at a time.

Recommended Post-Instrument Processing

1. Rinse slides in two changes of 95% ethanol to remove the leftover solution, followed by three changes of 100% ethanol.
2. Dehydrate slides in three changes of 100% xylene.
3. Coverslip with permanent mounting media.

Compatible with the VENTANA HE 600 system coverslipping protocol. For further information, refer to the VENTANA HE 600 system User Guide.

QUALITY CONTROL PROCEDURE

An example of a positive control material would be FFPE human tissue known to be glycogen rich, such as liver, which has been shown to contain glycogen in the hepatocyte cytoplasm.[2] Control tissue should be fresh autopsy, biopsy, or surgical specimen prepared or fixed as soon as possible in a manner identical to test sections. Such tissues should monitor all steps of the analysis, from tissue preparation through staining.

Use of a tissue section fixed or processed differently from the test specimen provides control for all reagents and method steps except fixation and tissue processing. The cellular components of other tissue elements may serve as the negative control.

Optimal laboratory practice is to include a positive control section on the same slide as the test tissue. This helps identify any failures applying reagents to the slide. Control tissue may contain both positive and negative staining elements and serve as both the positive and negative control.

The control tissue must be tested with each run.

Known positive tissue controls should only be utilized for monitoring the correct performance of processed tissues and test reagents, not as an aid in formulating a specific diagnosis of patient samples.

If the positive tissue components fail to demonstrate positive staining, results with the test specimens should be considered invalid. If the negative components demonstrate positive staining, results with patient specimens should also be considered invalid.

Unexplained discrepancies in control results should be referred to the local support representative immediately. If quality control results do not meet specifications, patient results are invalid. The cause must be identified and corrected, and the patient samples repeated.

STAINING INTERPRETATION / EXPECTED RESULTS

The Diastase Kit, used in conjunction with the PAS Staining Kit, is tested to demonstrate an absence of glycogen.

- Absence of glycogen: a trace to no magenta present
- Nuclei: blue to purple

In practice, two sections of the same tissue sample are stained with the PAS Staining Kit: one of them is treated with Diastase in advance, which results in two PAS-stained sections, one with and one without glycogen, that are then compared side-by-side to determine the presence and distribution of glycogen in the sample. Refer to the PAS Staining Kit method sheet for PAS staining interpretation.

SPECIFIC LIMITATIONS

Only positively charged microscope slides have been used and validated for this assay.

PERFORMANCE CHARACTERISTICS

ANALYTICAL PERFORMANCE

Staining tests for sensitivity, specificity, and precision were conducted and the results are listed below.

Sensitivity and Specificity

Analytical sensitivity and specificity was evaluated for normal tissue cases. All evaluated tissue cases (61/61) passed for acceptable staining as shown in Table 3.

Table 3. Sensitivity/Specificity of Diastase Kit was determined by testing the following FFPE normal tissues.

Tissue	# passed / # tested
Liver	51 / 51
Muscle	10 / 10

Precision

Precision of Diastase Staining Kit was determined across multiple runs, days, instruments, and reagent lots using multiple cut slides from 6 normal liver tissue cases. All acceptance criteria were fully met. Precision studies were performed for the Diastase Staining Kit according to Table 4.

Table 4. Precision slide studies for Diastase Kit.

Parameters Tested	# of conditions	# slides passed / # tested
Run to Run	3 runs, same day	54 / 54
Day to Day	5 days	88 / 90
Instrument to Instrument	3 instruments	53 / 54
Intra Run	same day, same instrument	54 / 54
Lot to lot	3 lots	54 / 54

The results demonstrated no significant difference in staining intensity among the slides.

TROUBLESHOOTING

1. Section thickness may affect quality and intensity of staining. If staining is inappropriate, contact your local support representative for assistance.
2. Necrotic or autolyzed tissue may exhibit nonspecific staining.
3. If the positive control is negative, tissue may have been improperly collected, fixed, or deparaffinized. Follow the proper procedure for collection, storage, and fixation.
4. If the positive control is negative, check that the slide has the proper barcode label. If the slide is labeled properly, check the other positive controls from the same run to determine if the controls were properly stained.
5. If excessive background staining occurs: incomplete paraffin removal could cause staining artifacts or no staining. If all paraffin is not removed from the slide, repeat the staining run using the extended deparaffinization option, if available.
6. Extended stay of the slides on-board the instrument after run completion will affect the quality and intensity of the staining. If the staining is inappropriate, remove slides promptly at the end of the run.
7. If tissue sections wash off the slide, confirm the slides are positively charged.
8. For corrective action, refer to the Instructions for Use section, the instrument User Guide or contact your local support representative.

REFERENCES

1. Layton C, Bancroft JD. Bancroft's Theory and Practice of Histological Techniques. In: Elsevier; 2019. Accessed 02/15/2021.
2. Carson F, Hladik C. Histotechnology: A Self Instructional Text, 3rd edition. Hong Kong: American Society for Clinical Pathology Press; 2009.
3. Clinical and Laboratory Standards Institute (CLSI). CLSI Web site. http://www.clsi.org/. Accessed November 3, 2011.
4. Occupational Safety and Health Standards: Occupational exposure to hazardous chemicals in laboratories. (29 CFR Part 1910.1450). Fed. Register.
5. Directive 2000/54/EC of the European Parliament and Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work.

NOTE: A point (period/stop) is always used in this document as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Ventana uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

GTIN — Global Trade Item Number

UDI — Unique Device Identification

Indicates the entity importing the medical device into the European Union

INTELLECTUAL PROPERTY

Additions, deletions or changes are indicated by a change bar in the margin.

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