Acu-Stain™ Mouse HRP Kits

Cat No.	Quantity
52-0001	15 mL Ready-To-Use
54-0001	100 mL Ready-To-Use

Intended Use

For In Vitro Diagnostic Use Only.

This kit is intended for use with Mouse Primary Antibody and other ancillary reagents supplied by user for qualitative detection of targeted protein (antigen) using immunohistochemistry (IHC) methodology by light microscopy on routine formalin-fixed, paraffin-embedded (FFPE) tissue section.

Interpretation of any positive or negative staining shall be supported by implementation of a proper control, and must be made within the context of the patient's clinical history and other diagnostic test by a qualified pathologist.

Summary And Explanation

This kit utilizes a biotinlyated secondary antibody to locate where the mouse primary antibody is bound to the target antigen. Streptavidin-HRP (horseradish peroxidase) is then applied and binds to the secondary antibody. This complex is observed through the use of a substrate-chromogen solution, which when added, results in a colored precipitate at the antigen location. The staining location and pattern is easily observable by light microscopy.

Reagents Supplied

Reagent A: One bottle of ready-to-use **Blocking Solution**, 10% Normal Goat Serum (non-immune)
In phosphate buffered saline (PBS) containing 0.09% Sodium Azide

Reagent B: One bottle of ready-to-use **Biotinylated Goat anti-Mouse Secondary Antibody**
In PBS containing stabilizing proteins and 0.09% Sodium Azide

Reagent C: One bottle of ready-to-use **Streptavidin-Peroxidase (HRP)**
In Tris Buffer containing stabilizing proteins and anti-microbial agents

Storage

Store at 2-8°C. Do not freeze.
All performance claims are void after the kit expiration date.

Materials Required But Not Supplied

Primary Antibody (Genemed offers prediluted and concentrate Mouse Monoclonal Primary Antibodies that are Ready-To-Use with Acu-Stain™ Mouse HRP Kits)

Primary Antibody Diluent (Cat No. 10-0001)

Negative Reagent Control (Non-immune Mouse IgG Cat No. 60-0045)

Positive and Negative Control Specimens

Microscope Slides, Positively Charged

Xylene

Ethanol

Endogenous Peroxidase Blocking Solution - 3% Hydrogen Peroxide

Wash Buffer - 10 mM Phosphate Buffer Saline, pH 7.4 ; optional with 0.05% Tween 20

HRP Substrate/Chromogen Reagents – AEC Substrate (Cat No. 10-0005)
– DAB Substrate (Cat No. 10-0006)

Hematoxylin (Cat No. 10-0027)

Antigen retrieval reagents (e.g. Cat No. 10-0022 Citrate Buffer pH 6.0 1X; Cat No. 10-0020 Citrate Buffer pH 6.0 20X; Cat No. 10-0024 Proteinase K)

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CE

Precautions

For professional users only.

Proper handling of this product as with any product derived from biological sources should be used according to local and applicable regulations.

Sodium Azide (NaN_3) is a toxic chemical and is present as an antimicrobial agent in Reagent A and B. The concentration in these products is not classified as hazardous. However, the build-ups of Sodium Azide may react with lead and copper plumbing to form highly explosive metal azides. Flush any disposed reagent with large volume of water to prevent azide build-up.

Sodium Azide inhibits peroxidase activity. Use caution when handling Streptavidin-HRP (Reagent C) to prevent any contamination with other reagents containing Sodium Azide.

Procedural Notes

The reagents for this kit have been designed to be Ready-To-Use. Any dilutions or alterations of the kit reagents may give erroneous staining results.

The directions accompanying this kit provide step by step instructions for optimal staining. Any change in procedure or incubation times may give erroneous staining results.

All reagents should be allowed to equilibrate at room temperature readily before usage. All incubations should be performed at room temperature in a humid environment.

Do not allow the section to dry out at any point in the staining procedure. The reagents are for single use.

Preliminary Preparation Of Slides

Routine de-paraffinization and rehydration of tissue section.
Antigen retrieval as required by the primary antibody.

Control Slides

Three types of control slides are necessary for proper interpretation.
Positive Tissue Control – A tissue containing the desired antigen.
Negative Tissue – A tissue that does not contain the desired antigen.
Reagent Control – A slide to be treated with a homologous non-immune immunoglobulin (Cat No. 60-0045).

Staining Protocol

Step 1: Endogenous Peroxidase Blocking

a) Submerge slides in Peroxidase Blocking Solution for 10 minutes.
b) Wash slides with Wash Buffer to remove excess Peroxidase Blocking Solution.
c) Tap off excess liquid and carefully wipe around tissue.

Step 2: Serum Blocking (Reagent A)

a) Add 2 drops (100 µL) or as much as needed of Serum Blocking Solution to completely cover each tissue section.
b) Incubate for 10 minutes.
c) Tap off excess liquid and carefully wipe around tissue. DO NOT RINSE.

Step 3: Primary Antibody Incubation

a) Prepare Primary Antibody to optimum concentration. If necessary, dilute with Primary Antibody Diluent.
b) Add 2 drops (100 µL) or as much as needed of Primary Antibody to completely cover each tissue.
c) Incubate for 30-60 minutes.
d) Rinse 3 times with Wash Buffer for 2 minutes each.
e) Tap off excess liquid and carefully wipe around tissue.

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Step 4: Biotinlyated Goat Secondary Antibody Incubation (Reagent B)

a) Add 2 drops (100 µL) or as much as needed of Biotinlyated Secondary Antibody to completely cover each tissue.
b) Incubate for 10 minutes.
c) Rinse 3 times with Wash Buffer for 2 minutes each.
d) Tap off excess liquid and carefully wipe around tissue.

Step 5: Enzyme Conjugate Incubation (Reagent C)

a) Add 2 drops (100 µL) or as much as needed of Enzyme Conjugate to completely cover each tissue.
b) Incubate for 10 minutes.
c) Rinse 3 times with Wash Buffer for 2 minutes each.
d) Tap off excess liquid and carefully wipe around tissue.

Step 6: Substrate/Chromogen

a) Perform Substrate/Chromogen incubation according to manufacturer's instruction.

Step 7: Counterstaining

a) Counterstain with Hematoxylin according to manufacturer's instruction.

Step 8: Mounting

a) Mount and coverslip the specimen with appropriate mounting solution based on substrate/Chromogen used.

Interpretation Of Staining Results

Step 1: Review Positive and Negative Controls. Do not proceed to next step if the staining intensity does not meet requirements.

Step 2: Score the tested specimens.

	Positive Control Tissue	Negative Control Tissue	Reagent Control	Test Tissue	Analysis of Result
1	+	--	--	+	Specimen contains the antigen
2	+	--	--	--	Specimen does not contain the antigen

Troubleshooting

	Positive Control Tissue	Negative Control Tissue	Reagent Control	Test Tissue	Analysis of Result
1	--	--	--	--	No staining
2	Weak +	--	--	+/-	Weak staining
3	+	+	+	+	High background staining

Possible causes and suggested action for: No staining on any slide.

1. Reagents not used in correct order
   → Repeat procedure following Staining Protocol Instructions
2. Substrate-Chromogen reagent not prepared properly
   → Prepare a fresh Substrate-Chromogen solution following the instructions included with the product
3. Primary antibody incubation steps were omitted or dilution was incorrect or wrong antibody was used
   → Repeat procedure following Staining Protocol Instructions using incubation times specified
   → Repeat procedure using correct dilution for primary antibody or correct primary antibody
4. Wrong Pretreatment
   → Repeat procedure using correct pretreatment

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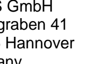

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Possible cause and suggested action for: Weak staining on all slides

1. Substrate-Chromogen reagent has expired
   → Prepare a fresh Substrate-Chromogen solution following the instructions included with the product
2. Incubation times were not long enough
   → Repeat procedure following Staining Protocol Instructions using incubation times specified
3. Specimen retained too much liquid after rinsing steps
   → Tap off excess liquid and carefully wipe around specimen after rinsing steps
4. Peroxidase Enzyme Conjugate (Reagent C) exposed to Sodium Azide
   → Use buffer without Sodium Azide, or check if Reagent C is contaminated with Sodium Azide during use or aliquot/pipetting
5. Primary antibody dilution was incorrect
   → Repeat procedure following Staining Protocol Instructions using incubation times specified
6. Insufficient Pretreatment
   → Repeat procedure using correct pretreatment

Possible cause and suggested action for: High background staining on all slides

1. Specimens contain high endogenous peroxidase activity
   → Check preparation of Peroxidase Solution and verify timing of specimens submerged in solution
2. Inadequate rinsing of slides
   → Use freshly prepared buffer solutions. Follow rinsing instructions specified
3. Deparaffinization not complete
   → Use fresh xylene. Check slides are deparaffinized before rehydration step
4. Over-reaction of substrate
   → Do not incubate substrate longer than specified in procedure
5. Specimens dry out during staining procedure
   → Incubate in humid environment. Wipe fewer than 10 slides at a time before adding next solution
6. Non-specific binding on tissue
   → Check use of Blocking Solution before Primary Antibody application
7. Wrong Pretreatment
   → Repeat procedure using correct pretreatment

Symbols

REF	LOT	IVD		
Catalog No.	Batch No.	In Vitro Diagnostic Use	Temperature Range	Use By

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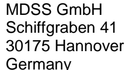

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