Power-Stain™ 1.0 Poly AP Kit for Mouse + Rabbit

Cat No.	Quantity
52-0020	15 mL
54-0020	100 mL

Intended Use

For In Vitro Diagnostic Use.

This kit is intended for use with Mouse and Rabbit Primary Antibodies 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 is a non-biotin system and utilizes a Poly AP (alkaline phosphatase) conjugate to locate where the mouse or rabbit primary antibody is bound to the target antigen. The complex formed between Poly AP conjugate and the mouse or rabbit primary antibody 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

One bottle of ready-to-use **Poly AP Conjugate for Mouse + Rabbit** in an enzyme conjugate 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 Primary Antibodies)
Primary Antibody Diluent (Cat No. 10-0001)
Reagent Control (Non-immune Mouse IgG Cat No. 60-0045 and Non-Immune Rabbit IgG Cat No. 60-0060)
Positive and Negative Control Specimens
Microscope Slides, Positively Charged
Xylene
Ethanol
Wash Buffer - 10 mM Phosphate Buffer Saline, pH 7.4; optional with 0.05% Tween 20
AP Substrate/Chromogen Reagents
Hematoxylin (Cat No. 10-0027, 10-0049)
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. 10-0021 Tris Buffer pH 9 20X; Cat No. 10-0023 Tris Buffer pH 9 1X; Cat No. 10-0046 Tris EDTA Buffer pH 9 1X; Cat No. 10-0037 Tris EDTA Buffer pH 9 20X; Cat No. 10-0024 Proteinase K; Cat No. 10-0025 Trypsin; Cat No. 10-0050 Ficin)

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.

Procedural Notes

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. For optimal results, do not substitute the reagent provided in the kit.

Poly AP Conjugate shall be equilibrated to room temperature readily before usage. All incubations should be performed at room temperature in a humid environment.

Do not allow the tissue 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.

Genemed Biotechnologies, Inc
458 Carlton Ct.
South San Francisco, CA 94080, U.S.A.
Tel: 650-952-0110
Fax: 650-952-1060

EC REP
MDSS GmbH
Schiffgraben 41
30175 Hannover
Germany

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 or Cat No. 60-0060)

Staining Protocol

Step 1: 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 at room temperature.

d) Rinse 3 times with Wash Buffer for 2 minutes each.

e) Tap off excess liquid and carefully wipe around tissue.

Step 2: Poly AP Conjugate Incubation

a) Add 2 drops (100 µL) or as much as needed of Enzyme Conjugate to completely cover each tissue.

b) Incubate for 15 ± 1 minutes.

c) Rinse 3 times with Wash Buffer for 2 minutes each.

d) Tap off excess liquid and carefully wipe around tissue.

Step 3: Substrate/Chromogen

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

Step 4: Counterstaining

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

Step 5: Mounting

a) Mount and coverslip the specimen with appropriate mounting.

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

Genemed Biotechnologies, Inc
458 Carlton Ct.
South San Francisco, CA 94080, U.S.A.
Tel: 650-952-0110
Fax: 650-952-1060

MDSS GmbH
Schiffgraben 41
30175 Hannover
Germany

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.

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. Primary antibody dilution was incorrect.
   → Repeat procedure following Staining Protocol Instructions using incubation times specified.
   → Repeat procedure using correct dilution for primary antibody.
5. Insufficient Pretreatment.
   → Repeat procedure using correct pretreatment.

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

1. Inadequate rinsing of slides.
   → Use freshly prepared buffer solutions. Follow rinsing instructions specified.
2. De-paraffinization not complete.
   → Use fresh xylene. Check slides are de-paraffinized before rehydration step.
3. Over-reaction of substrate.
   → Do not incubate substrate longer than specified in procedure.
4. Specimens dry out during staining procedure.
   → Incubate in humid environment. Wipe fewer than 10 slides at a time before adding next solution.
5. Wrong Pretreatment.
   → Repeat procedure using correct pretreatment.

Symbols

				
Catalog No.	Batch No.	In Vitro Diagnostic Use	Temperature Range	Use By

Genemed Biotechnologies, Inc
458 Carlton Ct.
South San Francisco, CA 94080, U.S.A.
Tel: 650-952-0110
Fax: 650-952-1060

MDSS GmbH
Schiffgraben 41
30175 Hannover
Germany