PolyStain DS Kit - for Goat and Rat antibody on Human

Mouse tissue (BCIP/AEC)

NB-23-00114- 3(120 ml)

NB-23-00114- 2(36 ml)

NB-23-00114- 1(12 ml)

Neo-Biotech
183, avenue Georges Clémenceau – 92000 Nanterre

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For Goat and Rat antibody on Human Mouse tissue (BCIP/AEC)

NB-23-00114-1; NB-23-00114-2; NB-23-00114-3

Storage: 2-8ºC

INTENDED USE:

The PolyStain DS-GRt-Hu/Ms B Kit is designed to use with user supplied goat and rat primary antibodies to detect two distinct antigens on human/mouse tissue or cell samples. The kit has been tested on paraffin embedded human and mouse tissues. However, this kit can be used on frozen specimen and freshly prepared monolayer cell smears. Double staining is one of most common methods used in immunohistostaining that allows for revealing two distinct antigens in a single tissue. The PolyStain DS-GRt-Hu/Ms B Kit from NeoBiotech Labs supplies two polymer enzyme conjugates: HRP polymer anti-Goat IgG and AP polymer anti-Rat IgG with two distinct substrates/chromogen, AEC (red) and BCIP/NBT (purple). User will apply two enzyme conjugates onto the specimen sequentially. When two proteins are present a purple/red color will develop depending presence and location of the antigen the two colors should be distinct. If only the anti-goat antigen is present only the AEC chromogen will be present and if the anti-Rat antigen is present only the BCIP/NBT will be present. The PolyStain DS-GRt-Hu/Ms B Kit is non-biotin system avoiding endogenous biotin non-specific binding.

KIT COMPONENTS:

Component No.	Content	6mL Kit	36mL Kit	120mL Kit
Reagent 1	Goat HRP(AEC) Polymer (RTU)	6mL	18mL	60mL
Reagent 2A	AEC Substrate (20x)	1mL	2mL	3mL
Reagent 2B	AEC Chromogen (20x)	2mL	4mL	6mL
Reagent 2C	Hydrogen Peroxide (20x)	1mL	2mL	3mL
Reagent 3	DS-GRt Blocker (RTU)	6mL	18mL	60mL
Reagent 4	Rat Primer (RTU)	6mL	18mL	60mL
Reagent 5	Rat AP Polymer (RTU)	6mL	18mL	60mL
Reagent 6	BCIP/NBT (RTU)	15mL	18mL	70mL
Reagent 7	NeoMount Universal (RTU)	7mL	18mL	70mL

Gt=Goat, Rt =Rat

RECOMMENDED PROTOCOL:

1. Fixation: To ensure the quality of the staining and obtain reproducible performance, user needs to supply appropriately fixed tissue and well prepared slides.
2. Tissues must be adhered to the slide properly to ensure maximum quality staining.
3. Paraffin embedded sections must be deparaffinized with xylene and rehydrated with a graded series of ethanol before staining.
4. Cell smear samples should be made up to as much of a monolayer as possible to obtain satisfactory results.
5. Three control slides will aid the interpretation of the result: positive and negative tissue controls, reagent control (slides treated with Isotype control reagent).
6. Proceed with IHC staining: **DO NOT** let specimens or tissues dry from this point on.
7. The fixation, tissue slide thickness, antigen retrieval and primary antibody dilution and incubation time affect results significantly. Investigator needs to consider all factors and determine optimal conditions when interpreting results.
8. **Note:** We recommend TBS-T to be used as the wash buffer to get the highest sensitivity and clean background. Phosphate in the PBS-T may inhibit the activity of the alkaline phosphatase.

Note: 1X TBS-T =50mM Tris HCl, 150mM NaCl, 0.05% Tween-20 pH7.6. NeoBiotech sells 10xTBS-T for your convenience (**NB-23-00201**)

Equipment or material needed but not provided:

1. Equipment and material for deparaffinization, such as fume absorbing hood, etc.
2. Heat source (microwave or hot plate) for HIER and antigen retrieval buffers.
3. Thermometer; Beaker; Timer
4. Wash buffer: 0.01 M PBS with 0.5% Tween20, pH7.4; 1X TBS-T =50mM Tris HCl, 150mM NaCl, 0.05% Tween-20 pH7.6.
5. Peroxidase and alkaline phosphatase blocking buffer
6. 100% ethanol; 100% Xylene; Hematoxylin

Reagent	Staining Procedure	Incubation Time (Min.)
1. Peroxidase and Alkaline Phosphatase Blocking Reagent Not provided **Fast, easy and it will block endogenous alkaline phosphatase**	a. Incubate slides in peroxidase and alkaline phosphatase blocking reagent (NeoPure Dual Enzyme Block NB-23-00193 was Recommended) for 10 minutes b. Rinse the slide using 2 changes of distilled water.	10 min.
2. HIER Pretreatment: Refer to antibody data sheet.	a. Heat Induced Epitope Retrieval (HIER) may be required for primary antibody. Refer to antibody datasheet. b. Wash with PBS-T containing 0.05% Tween-20 or 1X TBS-T (**See note 8 above**); 3 times for 2 minutes each.	Up to 1 hour
3. Primary Antibody Mix: one Goat and one Rat antibody Supplied by user	**Note**: Investigator needs to optimize dilution prior to double staining. a. Apply 2 drops or enough volume of goat and rat primary antibody mixture to cover the tissue completely. Incubate in moist chamber for 30-60 min. Recommend 30min to shorten total protocol time. b. Wash with PBS-T containing 0.05% Tween-20 or 1X TBS-T; 3 times for 2 minutes each.	30-60min
4. Reagent 1 Goat HRP(AEC) Polymer (RTU)	a. Apply 1 to 2 drops (50-100µL) of **Reagent 1** Goat HRP (AEC) Polymer to cover each section. b. Incubate in moist chamber for 15 min. c. Wash with PBS-T containing 0.05% Tween-20 or 1X TBS-T; 3 times for 2 minutes each.	15 min.
5. Reagent 2A, 2B, 2C: **Reagent 2A:** AEC Substrate (20x) **Reagent 2B:** AEC Chromogen (20x) **Reagent 2C:** Hydrogen Peroxide (20x)	a. Add 1 drop (50µL) of **Reagent 2A** to 1mL distilled water. Mix well. Add 2 drops of **Reagent 2B** and 1 drop of **Reagent 2C** to diluted **reagent 1**. Mix well. Keep away from light and use within 1 hour. b. Apply 2 drops (100µL) or enough volume of pre-mixed AEC solution to completely cover the tissue. Incubate for 5-15min, observe appropriate color development. c. Rinse well with distilled water. **(AEC is alcohol soluble; do not dehydrate.)**	10 min
6. Reagent 3 DS-GRt Blocker (RTU)	a. Apply 1 to 2 drops (50-100µL) of **Reagent 3** (DS-GRt Blocker) to cover each section. b. Incubate in moist chamber for 10 min. c. Blot off solution. **DO NOT** Rinse	10 min
7. Reagent 4 Rat Primer (RTU)	a. Add 2 drops (100µL) or enough volume of **Reagent 4** (Rat Primer) to cover the tissue section b. Incubate at Room Temperature for 10-15minutes. c. Wash with PBS-T containing 0.05% Tween-20 or 1X TBS-T; 3 times for 2 minutes each.	10 – 15 min

8. Reagent 5 Rat AP Polymer (RTU)	a. Apply 1 to 2 drops (50-100µL) of **Reagent 5** (Rat AP Polymer) to cover each section. b. Incubate in moist chamber for 10-15 min. c. Wash with 1X TBS-T only; 3 times for 2 minutes each.	10-15min
9. Reagent 6: BCIP/NBT (RTU))	a. Apply 2 drops or enough volume of **Reagent 6** (BCIP/NBT) to completely cover tissue. Incubate for 10 min. b. Rinse thoroughly with distilled water. c. Wash with PBS-T containing 0.05% Tween-20 or 1X TBS-T; 3 times for 2 minutes each.	10 min
10. HEMATOXYLIN Not provided	a. Counterstain with 2 drops (100µL) or enough volume of hematoxylin to completely cover tissue. Incubate for 5 seconds. **DO NOT** over stain with hematoxylin. b. Rinse thoroughly with tap water for 1 minute. c. Put slides in PBS for 5 seconds to blue, DO NOT over blue. d. Rinse well in distilled or tap water for 1 minute	
11. Reagent 7: NeoMount Universal (RTU)	a. Apply 2 drops (100µL) or enough **Reagent 7** (NeoMount Universal) to cover tissue when tissue is wet. Rotate the slides to allow NeoMount Universal spread evenly. **DO NOT** coverslip. b. Place slides horizontally in an oven at 40-50°C for at least 30 minutes or leave it at room temperature until slides are thoroughly dried. Hardened NeoMount Universal forms an impervious polymer barrier to organic solvent. Do not use oil directly on the top of dried NeoMount Universal. To coverslip see protocol note 2	30min in 40- 50°C oven Or: overnight at room temperature

PROTOCOL NOTES:

1. The fixation, tissue slide thickness, antigen retrieval and primary antibody dilution and incubation time affect results significantly. Investigator needs to consider all factors and determine optimal conditions when interpreting the result.
2. NeoMount Universal is an aqueous-based mounting media for immunohistochemistry. It is used as the permanent mounting media for chromogen such as Permanent Red, AP-Red, AEC, and BCIP. NeoMount Universal does not use a coverslip. However, if you need to coverslip your tissue, after NeoMount Universal has dried, dip the slide in xylene (1 to 2 seconds), apply an organic mounting solution (such as NeoMount Perm, Cat# NB-23-00156), and place cover glass on the slide. Store slides after they have dried completely.

PRECAUTIONS:

Standard laboratory personal protective equipment should be worn: i.e. gloves, eye protection and appropriate lab coat.

FOR RESEARCH USE

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Work Sheet for NB-23-00114 Kit

We designed these work sheets to help you track of each step. When staining fails these sheets help our technical support staff to pinpoint the problem. To insure that all steps are done properly, we recommend that the user fill in the actual time of their experimental step and any variation. Results will vary if time recommendations are not followed. RTU translates to ready to use.

- Used for tester to check "√ "each step during the experiment
- Steps follow after de-paraffinization
- Refer to insert for details of each step

Protocol Step	NB-23-00114 Protocol	Experiment 1 Date:	Experiment 2 Date:	Experiment 3 Date:	Experiment 4 Date:
Step 1	Peroxidase & levamisole Block NB-23-00193 is recommended. User supplied				
Step 2	HIER if needed				
Step 3	Gt 1°Ab & Rb 1°Ab mixture (30-60 min.)				
Step 4	**Reagent 1** Goat HRP Polymer RTU (15min)				
Step 5	**Reagent 2A, 2B, &2C** AEC requires mixing. (10min)				
Step 6	**Reagent 3** DS-GRt Blocker RTU (10min) Do Not Rinse Tap off & go directly to step 7				
Step 7	**Reagent 4** Rat Primer RTU (10-15 min.)				

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Step 8	**Reagent 5** Rat AP Polymer RTU (10-15min) Wash with 1xTBS-T only				
Step 9	**Reagent 6** BCIP/NBT RTU (10 min)				
Step 10	**Counter stain** Supplied by user				
Step 11	**Reagent 7** NeoMount Universal RTU				

Testing result: