PolyStain DS Kit - for 2 Mouse antibody on Human tissue

(BCIP/AEC)

NB-23-00098-1 (12 ml)

NB-23-00098-2 (36 ml)

NB-23-00098-3 (120 ml)

Neo-Biotech
183, avenue Georges Clémenceau – 92000 Nanterre

Neo-Biotech
183, avenue Georges Clémenceau – 92000 Nanterre

PolyStain DS Kit - for 2 Mouse antibody on Human tissue (BCIP/AEC)

NB-23-00098-1; NB-23-00098-2; NB-23-00098-3

Storage: 2- 8ºC

INTENDED USE:

The PolyStain DS Kit is designed to use with user supplied two mouse antibodies to detect two distinct antigens on human tissue or cell samples. This kit has been tested in paraffin tissue. 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 allow revealing two distinct antigens in a single tissue1.

PolyStain DS Kit from NeoBiotech supplies two polymer enzyme conjugates: HRP polymer anti-Mouse IgG and AP polymer anti-Mouse IgG with two distinct substrates/chromogen, AEC (Red color, use with HRP polymer anti-Mouse IgG) and BCIP/NBT (Purple/Blue color, use with AP polymer anti-Mouse IgG).

PolyStain DS Kit is non-biotin system that avoids endogenous biotin non-specific binding.

KIT COMPONENTS:

Component No.	Content	12mL Kit	36mL Kit	120mL Kit
Reagent 1	HRP polymer anti-Mouse IgG (RTU)	6mL	18mL	60mL
Reagent 2	BCIP/NBT Solution (RTU)	6mL	18mL	60mL
Reagent 3A	DS-MM Blocker A (RTU)	6 mL	18mL	60 mL
Reagent 3B	DS-MM Blocker B (RTU)	6mL	18mL	60mL
Reagent 4	AP polymer anti-Mouse IgG (RTU)	6mL	18mL	60mL
Reagent 5A	AEC Substrate Buffer (20x)	1mL	1mL	3mL
Reagent 5B	AEC Chromogen (20x)	2mL	2mL	6mL
Reagent 5C	Hydrogen Peroxide (20x	1mL	1mL	3mL
Reagent 6	NeoMount Universal (RTU)	6mL	18mL	60mL

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. Tissue need to be adhered to the slide tightly to avoid tissue falling off.
3. Paraffin embedded section must be deparffinized with xylene and rehydrated with a graded series of ethanol before staining.
4. Cell smear samples should be made as much monolayer as possible to obtain satisfactory results.
5. Three control slides will aid the interpretation of the result: positive tissue control, reagent control (slides treated with Isotype control reagent), and negative control.
6. It takes about 30 minutes to dissolve Fast Red tablet into the substrate buffer. Make sure to start preparing Fast Red solution near the end of the secondary antibody incubation.
7. Proceed IHC staining: **DO NOT** let specimen or tissue dry from this point on.

Reagent	Staining Procedures	Incubation Time (Min.)
1. Peroxidase Blocking Reagent Not provided	a. Incubate slides in peroxidase blocking reagent (Ready-to-use 3% H_2O_2 solution) for 10 minutes. b. Rinse the slide using distilled water.	10 min.
2. HIER Pretreatment: Refer to antibody data sheet.	a. Heat Induced Epitope Retrieval (HIER) may be required for primary antibody suggested by vendor. b. Wash with PBS for 2 min., 3 times	
3. Preblock (optional)	For paraffin section, Improved formula saves the need for a preblock step. For frozen tissue, preblock may or may not be required depending on fixative. (Preblock catalogue No.: **NB-23-00169** was Recommended.)	
4. Mouse Antibody 1: Supplied by user	**Notes**: Investigator needs to optimize dilution and incubation times prior to double staining. a. Apply 2 drops or enough volume of mouse primary antibody 1 to cover the tissue completely. Incubate in moist chamber for 30-60 min. b. Rinse with PBS containing 0.05% Tween-20 for 2 min., 3 times	30 – 60 min

5. Reagent 1: HRP polymer anti-Mouse IgG(RTU)	a. Apply 2 drops (50ul) of Reagent 1 HRP polymer antiMouse IgG to cover each section. b. Incubate in moist chamber for 15 min. c. Rinse with PBS containing 0.05% Tween-20 for 2 min., 3 times	15 min
6. Reagents 2: BCIP/NBT Chromogen (Ready-to-use)	a. Apply 2 drops or enough volume of **Reagents 2 BCIP/NBT CHROMOGEN** to completely cover tissue. Incubate for 3- 10 min. b. Rinse thoroughly with distilled water	3-10 min
7. Reagent 3A: DS-MM Blocker	a. Apply 2 drops or enough volume of **Reagent 3A** DS-MM Blocker A to cover the tissue completely. Mix well on the slide and Incubate in moist chamber for 30 min. b. Rinse with PBS containing 0.05% Tween-20 for 2 min., 3 times.	30min
8. Reagent 3B: DS-MM Blocker	a. Apply 2 drops or enough volume of **Reagent 3B** DS-MM Blocker B to cover the tissue completely. Mix well on the slide and Incubate in moist chamber for 5 min. b. Rinse with PBS containing 0.05% Tween-20 for 2 min., 3 times.	5 min.
9. Mouse antibody 2: Supplied by user	**Notes**: Investigator needs to optimize dilution and incubation times prior to double staining. a. Apply 2 drops or enough volume of mouse primary antibody 2 to cover the tissue completely. b. Rinse with PBS containing 0.05% Tween-20 for 2 min., 3 times.	30 -60 min
10. Reagent 4: AP polymer anti-Mouse IgG (RTU)	a. Apply 1drop (50ul) of **Reagent 4 AP polymer anti-Mouse IgG** to cover each section. b. Incubate in moist chamber for 15 min. c. Rinse with PBS containing 0.05% Tween-20 for 2 min., 3 times.	15 min
11. Reagent 5A, 5B, 5C: **Reagent 5A:** AEC Substrate Buffer (20x) **Reagent 5B:** AEC Chromogen (20x) **Reagent 5C:** Hydrogen Peroxide (20x)	a. Add 1 drop (50µl) of **Reagent 5A** and 1 drop or 2 drops (for higher sensitivity and contrast) of **Reagent 5B** and 1 drop of **Reagent 5C** to 1ml distill water. 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 5-10 min, observe appropriate color development c. Rinse well with distilled water. **(AEC is alcohol soluble; do not dehydrate.)**	5 -10 min

12. HEMATOXYLIN Not provided	a. Counterstain with 2 drops (100µl) or enough volume of hematoxylin to completely cover tissue. Incubate for 10-15 seconds. b. Rinse thoroughly with tap water for 2-3 min c. Put slides in PBS until show blue color (about ½ - 1 min.) d. Rinse well in distilled water	
13.Reagent6 : NeoMount Universal	a. Apply 2 drops (100µl) or enough volume of **Reagent 6** to cover tissue when tissue is wet. Rotate the slides to allow NeoMount Universal spread evenly. **DO NOT** coverslip. b. 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.	30 min. 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 effect results significantly. Investigator needs to consider all factors and determine optimal conditions when interpret the result.
2. NeoMount Universal is water-based mounting medium for immunohistology. It may be used as the permanent mounting media. It does not need coverslip. However, if you need to coverslip, after NeoMount Universal dried, dip the slide in xylene and take out immediately.
   Apply NeoMount Perm (Permanent mount, Catalog No. **NB-23-00156**) on the tissue and place cover glass on the slide. Store it after dry completely.

PRECAUTION:

DAB may be carcinogenic. Please wear gloves and take other necessary precautions.

FOR RESEARCH USE ONLY

Neo-Biotech
183, avenue Georges Clémenceau – 92000 Nanterre

Neo-Biotech
183, avenue Georges Clémenceau – 92000 Nanterre