PureStain Human-on-Human Kit, AP with Permanent Red

For Detection of Human Primary Antibodies on Human Tissues, Biotin Free

NB-23-00085-1 (18 ml)

NB-23-00085-2 (6 ml)

NB-23-00085-3 (110ml)

Neo-Biotech
183, avenue Georges Clémenceau – 92000 Nanterre

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PureStain Human-on-Human Kit, AP with Permanent Red

NB-23-00085-1; NB-23-00085-2; NB-23-00085-3

Storage: 4-8ºC

INTENDED USE:

Antigen detection with primary antibody of the same species as the test tissue yields high background when indirect detection method is used. This severely limits the use of screening human antibody on human tissues. NeoBiotech Labs PureStain Human-on-Human Detection System is designed for generating staining with the alkaline phosphatase (AP) enzyme of human primary antibodies on human tissues without background staining. The PureStain Human-on-Human Detection kit provides special blocking buffers, polymeric AP-linked secondary antibody as well as human primer in a ready to use system. This technology requires an overnight pre-incubation with primary antibody that results in excellent sensitivity and high specificity. It is a biotin-free system, therefore, overcomes the non-specific staining caused by streptavidin/biotin system due to endogenous biotins.

Note: This kit is recommended for cytoplasmic and membrane bound antigens other patterns of staining have not tested.

KIT COMPONENTS:

Component No	Content	6mL Kit	18mL Kit	110ml Kit
Reagent 1	Human Primer (RTU)	6mL	18mL	110mL
Reagent 2	Quenching Buffer (5x)	1.5mL	2.3mLx2	13mLx 2
Reagent 3	Hu Blocking A (RTU)	6mL	18mL	110mL
Reagent 4	Hu Blocking B (RTU)	6mL	18mL	110mL
Reagent 5	Human AP Polymer (RTU)	6mL	18mL	110mL
Reagent 6A	Permanent Red Substrate (RTU)	7mL	18 mL	Not Included
Reagent 6B	Permanent Red Activator (5x)	1.4mL	2 x 1.8mL	Not Included
Reagent 6C	Permanent Red Chromogen (100x)	70µL	180µL	Not included

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 needs to be adhered to the slide tightly to avoid tissue falling off.
3. Paraffin embedded section must be deparaffinized with xylene and rehydrated with a graded series of ethanol before staining.
4. Cell smear samples should be made into a monolayer as much as possible to obtain satisfactory results.
5. Three control slides will aid the interpretation of the result: positive tissue control, reagent control (slide treated with Isotype control reagent), and negative control.
6. Start staining procedures: **DO NOT** let specimen or tissue dry from this point on.
7. 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 inhibitor the activity of the alkaline phosphatase. **Note:** 1X TBS-T =50mM Tris HCl, 150mM NaCl, 0.05% Tween-20 pH7.6
8. Serum blocking before primary antibody incubation for NeoBiotech's PolyStain-1, PolyStain-2, and PolyStain-2Plus is not required because all our antibody conjugates are absorbed to human serum.

Reagent	**Staining Procedures Day 1** **Primary Human Antibody Preparation**	**Incubation Time (Min.)**
Dilute primary antibody in Reagent 1 Human Primer (RTU)	**Reagent 1** (Human Primer) is at ready to use concentration. Dilute your human primary antibody in the Human Primer at user determined primary antibody concentration. Mix gently for 30sec to 1min. Recommend only diluting amount needed for experiment. Place at 4C overnight.	O/N at 4C
Reagent **Prepare slides**	**Staining Procedures Day 2** See Recommended Protocols above	
1. Phosphatase blocking reagent: Supplied by user. We recommend using NeoBiotech Labs NeoPure Dual Enzyme Block **NB-23-00193** which blocks both endogenous phosphatase and peroxidase enzymes.	a. Apply 2 drops or enough volume of phosphatase blocking reagent (**NeoPure Dual Enzyme Block NB-23-00193)** to cover the tissue section and incubate b. Rinse the slide using distilled water move to pretreatment step. No Pretreatment then do step c. c. Wash 1X TBS-T for 2 minutes, 3 times. (See note 7 for TBS-T ingredients in recommended protocol above.)	10 min.

2. HIER Pretreatment: Refer to antibody supplier's data	a. Heat Induced Epitope Retrieval (HIER) may be required for primary antibody suggested by vendor b. Wash 1X TBS-T for 2 minutes, 3 times. (See **note 7** for TBS-T ingredients in recommended protocol above.)	
3. Bring to Room temp (Hu primary Ab diluted in Reagent 1) add Reagent 2 (Quenching Buffer 5x Concentration)	Remove Hu primary Ab diluted in **Reagent 1** from fridge and allow mix to come to room temperature. a. After Hu primary Ab diluted in **Reagent 1** has come to room temperature add Reagent 2 into mixture. b. Take the total volume of (Hu primary Ab diluted in Reagent 1) _____µl ÷ 5 = _____ µl amount of Reagent 2 (Quenching Buffer 5x Concentration). Incubate at room temperature for 15-30 min. c. Store on ice until you reach step 6. **Note**: Do not to quench for longer than 1 hour	15 - 30 min
4. Reagent 3: Hu Blocking A (RTU)	a. Add 2 drops or enough volume of **Reagent 3** (Hu Blocking A) to cover the tissue section completely and Incubate 30 min. b. Wash 1X TBS-T for 2 minutes, 3 times. (See **note 7** for TBS-T ingredients in recommended protocol above.)	30 min
5. Reagent 4: Hu Blocking B (RTU**)**	a. Add 2 drops or enough volume of **Reagent 4** (Hu Blocking B) to cover the tissue section completely and Incubate 5 min. b. Wash 1X TBS-T for 2 minutes, 3 times. (See **note 7** for TBS-T ingredients in recommended protocol above.)	5 min
6. Add Primary Ab mixture from step 3	**Note**: Optimized incubation time should be tested. We find that incubating 2-4 hours at room temperature or overnight at 4C works great without background. a. Add 2 drops or enough volume of mixture from step 3 {(Primary Ab) / (**Reagent 1** Human Primer) /(**Reagent 2** Quenching Buffer)} to cover the tissue section completely and Incubate 30-60 min. (Recommend 2 hours, but it will increase background) b. Wash 1X TBS-T for 2 minutes, 3 times. (See note 7 for TBS-T ingredients in recommended protocol above.)	30-60 min
7. Reagent 5: Human AP Polymer (RTU)	a. Apply 2 drops or enough volume of **Reagent 5** (Human AP Polymer) to cover the tissue section completely and incubate 10 minutes. b. Wash 1X TBS-T for 2 minutes, 3 times. (See note 7 for TBS-T ingredients in recommended protocol above.)	10 min.
8. Reagent 6A, 6B, 6C **Reagent 6A:** Permanent Red Substrate (RTU) **Reagent 6B:**	Note: Shake Permanent Red Activator before adding into Permanent Red Substrate. a. Add 200µL of **Reagent 6B** (Activator) into 1mL of **Reagent 6A** (Substrate buffer) and mix well. Add 10µL of **Reagent 6C** (Chromogen) into the mixture and mix well. [Note: For fewer slides, Add 100µL of **Reagent 6B** (Activator) into 500µL of	10 min + 10min

Permanent Red Activator (5x) **Reagent 6C:** Permanent Red Chromogen (100x) To get maximum sensitivity of AP polymer, Repeat the chromogen step	**Reagent 6A** (Substrate buffer) and mix well. Add 5µL of **Reagent 6C** (Chromogen) into the mixture and mix well. b. Apply 2 drops (100µL) or enough volume of Permanent Red working solution to completely cover the tissue. Incubate for 10 min, observe appropriate color development. To increase AP signa, aspirate or tap off chromogen and apply 2-3 drops (100µL) of the Permanent Red working solution again to completely cover the tissue for additional 5 to 10min. c. Rinse well with distilled water.	
9. Hematoxylin: Supplied by user	a. Counterstain with 2 drops or enough volume to cover tissue completely and wait about 10-20 seconds. b. Wash thoroughly under tap water for 1-2 min. c. Put slides in TBS not tween until show blue color (about 30-60 seconds) d. Rinse well in distilled water	
10. Mounting Medium User supply	Follow the manufacture data sheet procedure for mounting. Recommended product: 1. NeoMount AQ: Cat. No. **NB-23-00155-3** (18mL) 2. NeoMount Universal: Cat. No. **NB-23-00157-2** (18mL)	

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. Tissue staining is dependent upon the proper handling and processing of tissues prior to staining. Improper tissue preparation may lead to false negative results or inconsistent results.
3. Do not mix reagents from different lot.
4. Do not allow the slides to dry at any time during staining.

PRECAUTIOUS:

You should handle all kit components as potentially hazardous materials please wear gloves, eye protection, and appropriate lab entire in addition to lab coat when handling any or all reagents.

For research use only

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RELATED PRODUCTS:

Product	Catalog No.	Size		Product	Catalog No.	Size
PureStain Mouse-on-Mouse Kit, AP with Fast Red	NB-23-00073-5 NB-23-00073-4	6 ml 18ml		PolyStain 2-Step Plus Kit, HRP, Rat-NM, with AEC	NB-23-00064-3 NB-23-00064-2	6mL 18mL
PureStain Mouse-on-Mouse Kit, HRP with AEC	NB-23-00075-3 NB-23-00075-2	6 ml 18 ml		PolyStain 2-Step Plus Kit, AP, Rat-NM, with Permanent Red	NB-23-00070-2 NB-23-00070-3	6mL 18mL
PureStain Mouse-on-Mouse Kit, AP with Permanent Red	NB-23-00073-3 NB-23-00073-2	6mL 18mL		PolyStain 2-Step Plus Kit, HRP, Mouse-NR, with DAB	NB-23-00053-3 NB-23-00053-2	6mL 18mL
PureStain Mouse-on-Mouse Kit Blocking A & B solutions	NB-23-00076-4 NB-23-00076-2	100mL 18mL		PolyStain 2-Step Plus Kit, HRP, Mouse-NR, with AEC	NB-23-00065-3 NB-23-00065-2	6mL 18mL
PureStain Human-on-Human Kit, HRP with DAB	NB-23-00082-3 NB-23-00082-2 NB-23-00082-1	6mL 18mL 110mL		PolyStain 2-Step Plus Kit, AP, Mouse-NR, with Permanent Red	NB-23-00071-2 NB-23-00071-3	6mL 18mL
PureStain Human-on-Human Kit, HRP with AEC	NB-23-00083-2 NB-23-00083-1	6mL 18mL		PolyStain 2-Step Plus Kit, HRP, Rat-NM, with DAB	NB-23-00052-3 NB-23-00052-2	6mL 18mL

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