ab290116 – Rapid DAB Detection System

For use in the enrichment and purification of antibodies.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab290116

Storage and Stability

Store at 4ºC. Protect from light. Diluted reagents should be used promptly.

Materials Supplied

Item	Quantity
Tissue Primer	10 mL
Linker 1	10 mL
Linker 2	10 mL
Tracer	10 mL
Stable DAB/Plus Buffer	10 mL
Stable DAB/Plus Chromogen	15 mL
Empty Mixing Bottle	1 bottle (15 mL)

Materials Required But Not Supplied

- Pre-treatment reagents
- Controls
- Miscellaneous ancillary reagents

Reagent preparation

Preparation of Stable DAB/Plus Substrate Working Solution

1. Transfer 1 mL of the Stable DAB/Plus Buffer to a tube or mixing bottle.
2. Add 1 drop (approximately 20 µL) of Stable DAB/Plus Chromogen to the buffer. Mix thoroughly.
3. Working solution volume can be scaled up using the same ratio of buffer to chromogen.
4. Dispose of unused Stable DAB/Plus Substrate working solution in appropriate waste stream according to local, state, and federal regulations.

Recommended Staining Protocol – General Considerations

1. Paraffin embedded tissue sections must be deparaffinized with xylene or dewaxing agent and rehydrated with a graded series of ethanol and water washes before staining. Follow the standard dewaxing and rehydration protocol used in your lab.
2. The investigator needs to optimize the dilution and incubation times for primary antibodies.
3. Each immunostaining run should include known positive and negative controls to assure proper functioning of the staining system and aid in valid interpretation of the results.
4. Typical controls:
    a. Negative Controls
        i. Substitute normal, non-immune serum from the same host animal as the primary antibody (e.g. if using mouse monoclonal primary antibodies, use mouse non-immune serum).
        ii. Substitute matching host species isotype control for primary antibody.
        iii. Use antigen-absorbed primary antibody 9e.g. antibody reagent which has been adsorbed with the target antigen to remove specific antibody).
    b. Tissue Control
        i. A tissue known not to contain the target antigen.
5. Consult the primary antibody supplier for recommended antigen recovery treatments. Perform epitope recovery pre-treatments before starting the staining procedure.
6. Once the slide treatment has been started, DO NOT let tissues or specimens dry. This can cause undesirable background or artifacts.

Staining Protocol

1. Tissue Primer
    a. Apply the Tissue Primer and incubate – 1 minute.
    b. Rinse slides with 3 changes of wash buffer – 3 x 10 seconds.
2. Primary Antibody
    a. Incubate with Primary Antibody, prepared according to the manufacturer's recommended protocol at the desired concentration – 5 minutes.
    b. Wash slides with 3 changes of wash buffer – 3 x 10 seconds.
3. Linker 1
    a. Apply Linker 1 and incubate - 5 min.
    b. Rinse with 3 changes of wash buffer – 3 x 10 seconds.
4. Linker 2
    a. Incubate tissue with Linker 2 – 5 minutes.
    b. Wash slides 3 times with was buffer - 3 x 10 seconds.
5. Tracer
    a. Incubate the tissue with Tracer - 5 minutes.
    b. Wash slides with changes of wash buffer – 3 x 10 seconds.
6. Stable DAB/Plus
    a. Prepare Stable DAB/Plus substrate working solution as described in Reagent Preparation.
    b. Incubate tissue with prepared Stable DAB/Plus substrate. solution. Monitor level of staining to determine optimal incubation time - ~5 minutes.
    c. Rinse slides with 3 changes of water – 3 x 10 seconds.
7. Counterstain
    a. Incubate slides with counterstain (Hematoxylin) according to manufacturer's recommendation / standard lab protocol - ~ 1 minute.
    b. Wash slides 3 times with water – 3 x 10 seconds.
    c. Wash once with wash buffer - 1 x 10 seconds.

d. Wash one with water – 1 x 10 seconds

8. Dehydrate and coverslip
    a. Dehydrate tissues through graded ethanol series, followed by xylenes series.
    b. Apply coverslip with permanent mounting medium.

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)