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ACUITYAdvanced Protocol V.3

Sam Li[1]

[1]BioLegend

1 Works for me dx.doi.org/10.17504/protocols.io.8wbhxan

BioLegend

Sam Li
BioLegend

EXTERNAL LINK

http://www.biolegend.com/media_assets/support_protocol/ACUITYAdvanced_Protocol_052115.pdf

GUIDELINES

Tips:

1. Positively charged slides are recommended to securely adhere tissue.
2. Paraffin-embedded sections must be de-paraffinized with xylene and rehydrated with a graded series of ethanol before staining.
3. After de-paraffinization and rehydration, DO NOT let the specimen or tissue dry out.
4. Optimal working dilutions and incubation times are to be determined by the investigator.

FAQ:

- *For how long should I incubate with the chromagen?*
  -At least five minutes.

MATERIALS

NAME	CATALOG #	VENDOR
ACUITYAdvanced Biotin Free Polymer Detection System DAB Kit (Previously Covance catalog# SIG-32902)	931001	BioLegend
ACUITYAdvanced Biotin Free Polymer Detection System Kit (Previously Covance catalog# SIG-32904)	931101	BioLegend
ACUITYAdvanced Biotin Free Polymer Detection System Kit (Previously Covance catalog# SIG-32906)	931201	BioLegend

Peptide Exchange:

1 ACUITYAdvanced system is recommended for use on formalin fixed paraffin embedded sections.

2 Positively charged slides recommended to securely adhere tissue.

3 Paraffin embedded sections must be de-paraffinized with xylene and rehydrated with a graded series of ethanol before staining.

4 DO NOT let specimen or tissue dry from this point on. Optimal working dilution and incubation times are to be determined by the investigator.

Staining Protocol: Peroxidase Blocking

5 We recommend Peroxidase Block, Catalog#927401 or 927402. If supplied by user, prepare as per recommended protocol (supplied by user for 931101, and 931201).

6 When using ACUITYAdvanced hydrogen peroxide, incubate slides in 3% hydrogen peroxide blocking reagent for 10 minutes (hydrogen peroxide is provided with 931001).

7 Rinse with distilled water.

Heat Induced Epitope Retrieval (HIER) or enzymatic digestion

8 Please refer to your antibody datasheet for recommended protocols if required.

9 For HIER we recommend HIER, Catalog # 928502 (order separately). HIER or enzyme for digestion to be supplied by user.

10 Wash with PBS 2 minutes, 3 times.

ACUITYAdvanced Reagent 1 (Serum Block)

11 Apply 2 drops (100 µL or enough volume to cover tissue section) of ACUITYAdvanced Reagent

12 Incubate in a humidity chamber for 10 minutes.

13 Drain or blot off solution. **Do not rinse.**

Primary Antibody (supplied by user)

14 Apply 2 drops (100 µL or enough volume to cover tissue section) of primary antibody.

15 Incubate in a humidity chamber for 30-60 minutes.

16 Rinse with PBS 2 minutes, 3 times.

ACUITYAdvanced Reagent 2 (Boost)

17 Apply 2 drops (100 µL or enough volume to cover tissue section) of ACUITYAdvanced Reagent 2.

18 Incubate in a humidity chamber for 15-20 minutes.

19 Rinse with PBS 2 minutes, 3 times.

ACUITYAdvanced Reagent 3 (HRP Polymer)

20 Apply 2 drops (100 µL or enough volume to cover tissue section) of ACUITYAdvanced Reagent 3.

21 Incubate in a humidity chamber for 15 minutes.

22 Rinse with PBS 2 minutes, 3 times.

Chromogen (supplied by user for 931101, 931201)

23 If supplied by user; prepare as per recommended protocol.

24 When using ACUITYAdvanced Chromogens (provided with kit 931001), please reference Chromogen Preparation Table.

25 Rinse with distilled or tap water (AEC is alcohol soluble; do not dehydrate).

Counterstain and mount (supplied by user)

26 Counterstain with desired counterstain.

27 Mount and coverslip.

Chromogen Preparation

28 AEC chromogen should be prepared 1 part AEC chromogen to 50 parts AEC Substrate Buffer.

29 DAB chromogen should be prepared 1 part DAB Chromogen to 25 parts DAB Substrate Buffer. The following table provides some sample preparation examples.

ACUITY*Advanced* Chromogen Preparation Table

Chromogen	Desired Working Volume	Chromogen Volume	Buffer Volume
AEC	5 mL	0.1 mL AEC	5 mL
	10 mL	0.2 mL AEC	10 mL
	25 mL	0.5 mL AEC	25 mL
DAB	10 mL	0.4 mL DAB	10 mL
	20 mL	0.8 mL DAB	20 mL
	30 mL	1.20 mL DAB	30 mL

Intended Use: Research Use Only (RUO); This product is sold for laboratory research use only, not for human or *in vivo* use.

Warranty/Conditions: BioLegend products may not be resold or modified for resale without prior written approval.

Storage: Store between 2-8°C.