ACUITYAdvanced Protocol

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Abstract

ACUITYAdvanced Biotin Free HRP
Polymer Detection System for Immunohistochemistry

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Guidelines

ACUITY*Advanced* Kit Components

BioLegend Catalog Number	Previous Catalog Number	Number of Tests	Peroxidase Block (3% H_2O_2)	Reagent 1 (Serum Block	Reagent 2 (Boost	Reagent 3 (HRP Polymer)	DAB Chromogen	DAB Substrate Buffer	AEC Chromogen	AEC Substrate Buffer
930901	SIG-32900	50	5mL	5mL	5mL	5mL	---	---	1mL	24mL
931001	SIG-32902	50	5mL	5mL	5mL	5mL	2mL	24mL	---	---
931101	SIG-32904	150	---	15mL	15mL	15mL	---	---	---	---
931201	SIG-32906	500	---	50mL	50mL	50mL	---	---	---	---
930501	SIG-32910	700 Cap Gap	---	125mL	125mL	125mL	---	---	---	---
930601	SIG-32912	700 Cap Gap	125mL	125mL	125mL	125mL	6mL	2x125mL	---	---
930701	SIG-32914	10,000	1000mL	1000mL	1000mL	1000mL	100mL	2x1000mL	---	---

Protocol

Tissue Section Preparation

Step 1.

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

Tissue Section Preparation

Step 2.

Positively charged slides recommended to securely adhere tissue.

Tissue Section Preparation

Step 3.

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

Tissue Section Preparation

Step 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

Step 5.

We recommend Peroxidase Block, Catalog# 927401 or 927402. If supplied by user, prepare as per recommended protocol (supplied by user for 931101, 931201, 930501).

Staining Protocol - Peroxidase Blocking

Step 6.

When using ACUITYAdvanced hydrogen peroxide, incubate slides in 3% hydrogen peroxide blocking reagent
for 10 minutes (hydrogen peroxide is provided with 930901, 931001, 930601 and 930701).

Staining Protocol - Peroxidase Blocking

Step 7.

Rinse with distilled water.

Staining Protocol - Heat Induced Epitope Retrieval (HIER) or enzymatic digestion

Step 8.

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

Staining Protocol - Heat Induced Epitope Retrieval (HIER) or enzymatic digestion

Step 9.

For HIER we recommend HIER, Catalog # 928501 (order separately). HIER or enzyme for digestion to be supplied by user.

Staining Protocol - Heat Induced Epitope Retrieval (HIER) or enzymatic digestion

Step 10.

Wash with PBS 2 minutes, 3 times.

Staining Protocol -ACUITYAdvanced Reagent 1 (Serum Block)
Step 11.

A. Apply 2 drops (100 µL or enough volume to cover tissue section) of ACUITYAdvanced Reagent 1.

Staining Protocol -ACUITYAdvanced Reagent 1 (Serum Block)
Step 12.

Incubate in a humidity chamber for 10 minutes.

Staining Protocol -ACUITYAdvanced Reagent 1 (Serum Block)
Step 13.

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

Staining Protocol -Primary Antibody (supplied by user)
Step 14.

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

Staining Protocol -Primary Antibody (supplied by user)
Step 15.

Incubate in a humidity chamber for 30-60 minutes.

Staining Protocol -Primary Antibody (supplied by user)
Step 16.

Rinse with PBS 2 minutes, 3 times.

Staining Protocol - ACUITYAdvanced Reagent 2 (Boost)
Step 17.

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

Staining Protocol - ACUITYAdvanced Reagent 2 (Boost)
Step 18.

Incubate in a humidity chamber for 15-20 minutes.

Staining Protocol - ACUITYAdvanced Reagent 2 (Boost)
Step 19.

Rinse with PBS 2 minutes, 3 times.

Staining Protocol - ACUITYAdvanced Reagent 3 (HRP Polymer)

Step 20.

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

Staining Protocol - ACUITYAdvanced Reagent 3 (HRP Polymer)

Step 21.

Incubate in a humidity chamber for 15 minutes.

Staining Protocol - ACUITYAdvanced Reagent 3 (HRP Polymer)

Step 22.

Rinse with PBS 2 minutes, 3 times

Staining Protocol - Chromogen (supplied by user for 931101, 931201, 930501)

Step 23.

If supplied by user; prepare as per recommended protocol.

Staining Protocol - Chromogen (supplied by user for 931101, 931201, 930501)

Step 24.

When using ACUITYAdvanced Chromogens (provided with kits 930901, 931001, 930601 and 930701) please
reference Chromogen Preparation Table.

Staining Protocol - Chromogen (supplied by user for 931101, 931201, 930501)

Step 25.

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

Staining Protocol - Counterstain and mount (supplied by user)

Step 26.

Counterstain with desired counterstain.

Staining Protocol - Counterstain and mount (supplied by user)

Step 27.

Mount and coverslip.

Staining Protocol - Chromogen Preparation

Step 28.

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

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