Version 1d Last updated 16 March 2020

ab214749 Human on human IHC kit (HRP/DAB)

For staining human primary antibodies on human tissues without background staining.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

Abcam's Human on human IHC kit (HRP/DAB) (ab214749) is designed for staining human primary antibodies on human tissues without background staining.

Antigen detection using a primary antibody of the same species as the test tissue yields high background when an indirect detection method is used. This severely limits the ability to screen human antibodies on human tissues. Abcam's Human on human IHC kit (HRP/DAB) (ab214749) is a ready to use system that results in excellent sensitivity and high specificity using a simple protocol. Furthermore, as the protocol uses a polymer-based, biotin-free system, it does not require blocking of endogenous biotin. Detection is based on horseradish peroxidase (HRP) and DAB.

2. Precautions

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit.

We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.

Reagents should be treated as possible mutagens and should be handle with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.

Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.

All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

3. Storage and Stability

Store kit at +4°C immediately upon receipt. Kit has a storage time of 1 year from receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

4. Limitations

Assay kit intended for research use only. Not for use in diagnostic procedures.

Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

5. Materials Supplied

Item	Quantity			Storage Condition
	6 mL Kit	18 mL Kit	110 mL Kit	
Human Primer	6 mL	18 mL	110 mL	+4°C
5X Quenching Buffer	1.5 mL	2X 2.3 mL	2X 13 mL	+4°C
Blocking Buffer A	6 mL	18 mL	110 mL	+4°C
Blocking Buffer B	6 mL	18 mL	110 mL	+4°C
Human HRP Polymer	6 mL	18 mL	110 mL	+4°C
DAB Substrate Buffer	12 mL	2X 15 mL	120 mL	+4°C
20X DAB Chromogen	1.5 mL	2 mL	6 mL	+4°C

6. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Peroxidase blocking reagent (e.g. ab64218)
- Appropriately fixed tissue section
- 100% Xylene
- 100% Ethanol
- Control slides
- Distilled water
- PBS/0.05% Tween 20 (e.g. ab64247)
- PBS (e.g. ab64026)
- Hematoxylin (e.g. ab128990)
- Mounting medium (e.g. ab64230)

7. Technical Hints

Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.

Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.

Avoid foaming or bubbles when mixing or reconstituting components.

Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.

Ensure all reagents and solutions are at the appropriate temperature before starting the assay.

Make sure all necessary equipment is switched on and set at the appropriate temperature.

The fixation, tissue section thickness, antigen retrieval method and primary antibody dilution and incubation time affect results significantly. The investigator needs to consider all factors and determine optimal conditions when interpreting the results.

Tissue staining is dependent on the proper handling and processing of tissues prior to staining. Improper tissue preparation may lead to false negative results or inconsistent results.

Do not allow the slides to dry at any time during staining.

8. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use.
- Prepare only as much reagent as is needed on the day of the experiment.

8.1 Human Primer
Ready to use. Store at +4°C.

8.2 5X Quenching Buffer
Ready to use. Store at +4°C.

8.3 Blocking Buffer A
Ready to use. Store at +4°C.

8.4 Blocking Buffer B
Ready to use. Store at +4°C.

8.5 Human HRP Polymer
Ready to use. Store at +4°C.

8.6 DAB Substrate Buffer
Ready to use. Store at +4°C.

8.7 20X DAB Chromogen
Ready to use. Store at +4°C.

9. Recommended Preparation

- Briefly centrifuge small vials at low speed prior to opening

9.1 Fixation: To ensure the quality of the staining and obtain reproducible performance, the user needs to supply appropriately fixed tissue and well prepared slides.

9.2 Tissue needs to be adhered to the slide tightly to avoid the tissue falling off.

9.3 Paraffin embedded section must be deparaffinized with xylene and rehydrated with a graded series of ethanol before staining.

9.4 Cell smear samples should be made into a monolayer as much as possible to obtain satisfactory results.

9.5 Three control slides will aid the interpretation of the result: positive tissue control, reagent control (slide treated with Isotype control reagent), and negative control.

9.6 Once the staining procedure has been started, DO NOT let specimen or tissue dry.

10. Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use, unless stated otherwise.
- Prepare all reagents and samples as directed in the previous sections.

Day 1

10.1 Dilute the primary antibody in Human Primer at 4^{0}C to a user determined primary antibody concentration. Mix gently for 30 seconds to 1 minute. We recommend only diluting the amount needed for the experiment. Incubate at 4^{0}C overnight.

Day 2

10.2 Prepare the slide - see Recommended Preparation section.

10.3 Apply enough volume of peroxidase blocking reagent to cover the tissue section and incubate for 10 minutes.

10.4 Rinse the slide with distilled water.

10.5 Optional: perform pre-treatment step, if required. Heat Induced Epitope Retrieval (HIER) may be required – follow the primary antibody vendor's recommendations. If pre-treatment is not required, skip to 10.6.

10.6 Wash slide with PBS/0.05% Tween 20 for 2 minutes. Repeat two more times.

10.7 Allow the human primary antibody diluted in Human Primer to equilibrate to room temperature.

10.8 Add 5X Quenching Buffer to the mixture of human primary antibody and Human Primer. Calculate the volume of 5X Quenching buffer required using the following formula:

Amount of 5X Quenching Buffer (µL) = Total volume of primary antibody diluted in Human Primer (µL) ÷ 5

10.9 Incubate at room temperature for 15-30 minutes.

10.10 Store at 4^{0}C or on ice until step 10.15. Avoid quenching for longer than 1 hour. For example, if step 10.9 was performed for 15 minutes, do not store at 4^{0}C or on ice for longer than 45 minutes in Step 10.10.

10.11 Add 2 drops or enough volume of Blocking Buffer A to cover the tissue section completely and incubate for 30 minutes.

10.12 Wash with PBS/0.05% Tween 20 for 2 minutes. Repeat two more times.

10.13 Add 2 drops or enough volume of Blocking Buffer B to cover the tissue section completely and incubate for 5 minutes.
10.14 Wash with PBS/0.05% Tween 20 for 2 minutes. Repeat two more times.
10.15 Add 2 drops or enough of the primary antibody mixture from step 10.10 to cover the tissue section completely and incubate for 30-60 minutes. Incubation for 2 hours may be required but will result in increased background.

Δ **Note:** The optimal incubation time should be determined experimentally. Typically, good staining is obtained by incubating for 2-4 hours at room temperature or overnight at 4^0C.

10.16 Wash with PBS/0.05% Tween 20 for 2 minutes. Repeat two more times.
10.17 Apply 2 drops or enough volume of Human HRP Polymer to cover the tissue section completely and incubate for 10 minutes.
10.18 Wash with PBS/0.05% Tween 20 for 2 minutes. Repeat two more times.
10.19 Add 1 drop (or 2 drops for higher contrast) of 20X DAB Chromogen to 1 mL of DAB Substrate Buffer. Mix well. Protect from light and use within 7 hours.
10.20 Apply 2 drops (100 µL) or enough volume of the DAB chromogen/substrate mixture to completely cover the tissue and incubate for approximately 5 minutes. Monitor the development of color under amicroscope.
10.21 Wash with tap water for 1-2 minutes.
10.22 Counterstain with 2 drops or enough volume of hematoxylin to cover tissue completely and wait for 10-20 seconds.
10.23 Wash the slide thoroughly under tap water for 1-2 minutes.
10.24 Place the slide in PBS until the blue color has developed (about 30-60 seconds).
10.25 Rinse well in distilled water.
10.26 Follow the mounting medium vendor's protocol for mounting.

11. General IHC Troubleshooting

Problem	Cause	Solution
No Staining	The primary antibody and the secondary detection polymer are not compatible.	Use a primary antibody that was raised in a species that can be detected by the polymer detection system (e.g. goat primary antibody with goat AP polymer).
	Not enough primary antibody is bound to the protein of interest.	Use less dilute antibody, Incubate longer (e.g. overnight) at 4°C.
	The antibody may not be suitable for IHC procedures which reveal the protein in its native (3D form).	Test the antibody in a native (non-denatured) WB to make sure it is not damaged.
	The protein is not present in the tissue of interest.	Run a positive control recommended by the supplier of the antibody.
	Deparaffinization may be insufficient.	Deparaffinize sections longer, change the xylene.
	The primary/secondary antibody/amplification kit may have lost its activity due to improper storage, improper dilution or extensive freezing/thawing.	Run positive controls to ensure that the primary/secondary antibody is working properly.
	The protein of interest is not abundantly present in the tissue.	Use an amplification step to maximize the signal.
	Fixation procedures (using formalin and paraformaldehyde fixatives) may be modifying the epitope the antibody recognizes.	Use antigen retrieval methods to unmask the epitope, fix for less time.
	The protein is located in the nucleus and the antibody (nuclear protein) cannot penetrate the nucleus.	Add a permeabilizing agent to the blocking buffer and antibody dilution buffer.
	The PBS buffer is contaminated with bacteria that damage the phosphate groups on the target protein.	Add 0.01% azide in the PBS antibody storage buffer or use fresh sterile PBS.

Problem	Cause	Solution
High Background	Blocking of non-specific binding might be absent or insufficient.	Increase the blocking incubation period and consider changing blocking agent. Abcam recommends 10% normal serum 1hr for sections or 1-5% BSA for 30 min for cells in culture.
	Incubation temperature may be too high.	Incubate sections or cells at 4°C.
	The primary antibody concentration may be too high.	Titrate the antibody to the optimal concentration, incubate for longer but in more dilute antibody (a slow but targeted binding is best).
	The secondary detection polymer may be binding non-specifically (damaged).	Run a secondary polymer negative control without primary antibody.
	Tissue not washed enough, fixative still present.	Wash extensively in PBS between all steps.
	Endogenous peroxidases are active.	Use enzyme inhibitors i.e. Levamisol (2 mM) for alkaline phosphatase or H2O2 (0.3% v/v) for peroxidase.
	Fixation procedures (using formalin and paraformaldehyde fixatives) are too strong and modified the epitope the antibody recognizes.	Change antigen retrieval method, decrease the incubation time with the antigen unmasking solution.
	Too much substrate was applied (enzymatic detection).	Reduce substrate incubation time.
	The chromogen reacts with the PBS present in the cells/tissue (enzymatic detection).	Use Tris buffer to wash sections prior to incubating with the substrate, then wash sections/cells in Tris

		buffer.
	Pemeabilization has damaged the membrane and removed the membrane protein (membrane protein).	Remove permeabilizing agent from your buffers.
Non-specific staining	Primary/secondary polymer concentration may be too high.	Try decreasing the antibody concentration and/or the incubation period. Compare signal intensity against cells that do not express the target.
	Endogenous peroxidases are active.	Use enzyme inhibitors i.e. Levamisol (2 mM) for alkaline phosphatase or H_2O_2 (0.3% v/v) for peroxidase.
	The primary antibody is raised against the same species as the tissue stained (e.g. mouse primary antibody tested on mouse tissue). When the secondary antibody is applied it binds to all the tissue as it is raised against that species.	Use a primary antibody raised against a different species than your tissue.
	The sections/cells have dried out.	Keep sections/cells at high humidity and do not let them dry out.

12. Notes

Technical Support

Austria
wissenschaftlicherdienst@abcam.com | 019-288-259
France
supportscientifique@abcam.com | 01.46.94.62.96
Germany
wissenschaftlicherdienst@abcam.com | 030-896-779-154
Spain
soportecientifico@abcam.com | 91-114-65-60

Switzerland
technical@abcam.com
Deutsch: 043-501-64-24 | Français: 061-500-05-30
UK, EU and ROW
technical@abcam.com | +44(0)1223-696000

Canada
ca.technical@abcam.com | 877-749-8807
US and Latin America
us.technical@abcam.com | 888-772-2226

Asia Pacific
hk.technical@abcam.com | (852) 2603-6823
China
cn.technical@abcam.com | 400 921 0189 / +86 21 2070 0500
Japan
technical@abcam.co.jp | +81-(0)3-6231-0940
Singapore
sg.technical@abcam.com | 800 188-5244

Australia
au.technical@abcam.com | +61-(0)3-8652-1450
New Zealand
nz.technical@abc.com | +64-(0)9-909-7829