

**LinBlock**

Ready-to-use

**CatNo** **RAG0149UK**

Quantity: 100 ml  
 Lot: See label  
 Expiration Date: See label  
 Storage: +2 - +8°C  
 Note: DO NOT FREEZE!

Additional CatNo	Description	Notes
RAG0149LK	20 ml LinBlock, sufficient for approx. 100 applications	
RAG0149PK	50 ml LinBlock, sufficient for approx. 250 applications	
RAG0149UK	100 ml LinBlock, sufficient for approx. 500 applications	

**Background:**

The use of antibodies from the same species is a common problem in immunohistochemical double staining. The "LinBlock" reagent removes the antibodies from the first staining and avoids cross reactions with the second staining. Besides the removal of the antibodies and also the simultaneous blocking of the enzyme system allows the use of two chromogenes of the same enzyme system. This application is most suitable when the antigen expression is localized on different cell membranes. A basic rule is to use the antibody always first which gives the best and clearest reagent product in single staining. Preferably also the antigen with the lowest expression should be stained first.

The LinBlock can be used for cryostat as well as paraffin sections.

There is a limit to each method: The antigen expression in the same cell e.g. nuclear/cytoplasmic or the localization of both antigens on the same cell surface often require different enzyme systems for a successful application. Thereby the antibodies should be used in the order of staining from the center outwards.

LinBlock was developed to make technical conditions easier and to improve results of double staining procedures.

**Volume/Amount:**

100 ml

**Effectiveness:**

LinBlock has two functions:

It eliminates the antibodies of the first staining and inhibits the enzymes.

**Form:**

Ready to use

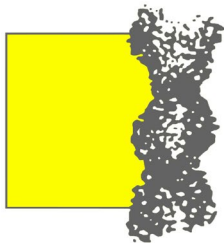
**Notes**

Multiple labeling often facilitates the interpretation of immunohistochemical staining. For this purpose the following basic rules should be observed:

- As far as possible two different enzyme systems should be used, when these systems were applied successfully in single staining.
- Effective double staining depends on the quality of the single staining.
- Double staining can never be superior to single staining.
- Normally monoclonal antibodies give a clearer result than polyclonal antibodies.
- Staining artifacts of single staining add up during multiple staining.
- The more staining per section the more difficult it is to get a good result.
- Combine only substrates which can be mounted with the same mounting medium.

RAG0149UK-230110-1/3

**For Research purposes only. Not for therapeutic or diagnostic use.**



## LinBlock

CarNo **RAG0149UK**

### Application Protocol

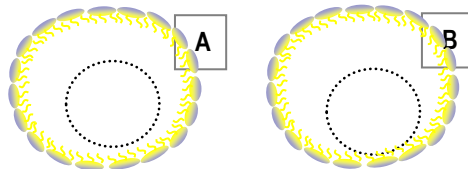
**Requirement:**

Start single staining with all antibodies, which you would like to use in multiple staining. These single stainings must produce a clear and definable reaction product. Only then you can perform multiple staining.

**Application:**

At the end of the first staining wash the sections before adding **LinBlock** with TBS-buffer (0.05 M TRIS-Base, 0.15 M NaCl, pH 7.2) or PBS by Dulbecco (DPBS) (LINARIS CatNo. GBP1622AL powder). Excess buffer should be removed carefully. Then incubate the sections with **LinBlock** at room temperature for 2 x 2 minutes. Add adequate **LinBlock** on the sections (2 drops, approx 100µl) so the tissue is completely covered. Wash the sections briefly with double distilled water and incubate again with the **LinBlock**. Afterwards wash the sections for 2 x 5 minutes with TBS or DPBS-buffer. Now the sections are ready for the incubation with the next primary antibody.

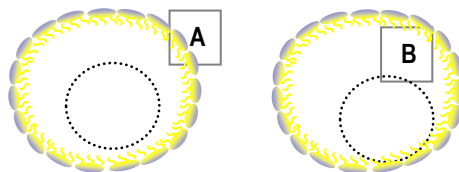
**Antigen localization on different membrane surface**



Recommended substrate:

1. Staining A: DAB or Vector NovaRed™
2. Staining B: Ni/Co-DAB, HistoGreen or Vector VIP

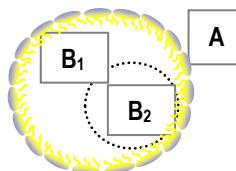
**Antigen localization on different cells in different cell compartments.**



Recommended substrate:

1. Staining A: DAB (HRP)
2. Staining B: HistoGreen (HRP)

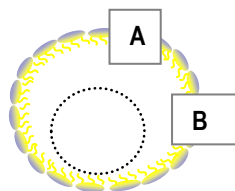
**Antigen localization in the same cell.**



Recommended substrate:

1. Staining B1 or B2: VectorRed (AP)
2. Staining A: DAB (HRP)

**Antigen localization on the same cell.**

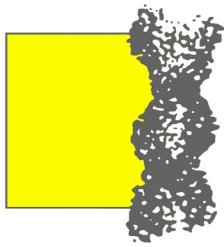


Recommended substrate:

1. Staining A: DAB (HRP)
2. Staining B: VectorRed (AP)

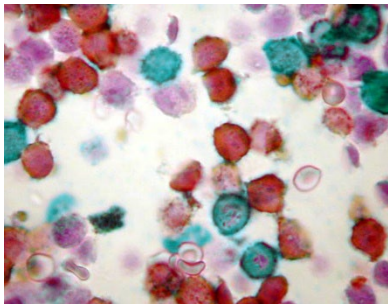
RAG0149UK-230110-2/3

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**LinBlock**

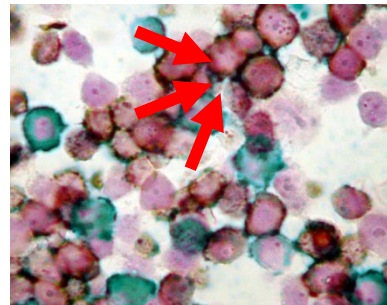
CatNo **RAG0149UK**



**With LinBlock**

Anti-human CD4 stained with Vector NovaRed (Vector-CatNo SK-4800)

anti-human CD8 stained with HistoGreen (Linaris CatNo E109)



**Without LinBlock**

Anti-human CD4 stained with Vector NovaRed (Vector CatNo SK-4800)

anti-human CD8 stained with HistoGreen (Linaris CatNo E109)

**Application Protocol**

Mounting	Name	Linaris CatNo	Aqueous mounting	Permanent mounting	Depending on what mounting medium was chosen only suitable substrates should be used. The scheme on the left side should make it easier for you to choose.
	HRP:DAB	SK-4100	x	x	
	HRP:Vector NovaRed	SK-4800	x	x	
	HRP:HistoGreen	E109		x	
	HRP:ViP	SK-4600	x	x	
	HRP:Vector SG	SK-4700	x	x	
	HRP:AEC	SK-4200	x		
	HRP:LinRed	E201	x		
	AP:VectorRed	SK-5100	x	x	
	AP:VectorBlue	SK-5300		x	
	AP:VectorBlack	SK-5200		x	
<b>Storage:</b>	Store at 2 -8°C. <b>DO NOT FREEZE!</b>				
<b>Shelf Life:</b>	12 month from date of despatch.				

RAG0149UK-230110-3/3

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