Datasheet for ABIN101961

Guinea Pig anti-Rabbit IgG (Heavy & Light Chain) Antibody - Preadsorbed

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Overview

Quantity:	200 µL
Target:	IgG
Binding Specificity:	Heavy & Light Chain
Reactivity:	Rabbit
Host:	Guinea Pig
Clonality:	Polyclonal
Application:	ELISA, Immunohistochemistry (IHC), Western Blotting (WB), Cleavage Under Targets and Tagmentation (CUT&Tag), Cleavage Under Targets and Release Using Nuclease (CUT&RUN)

Product Details

Purpose:	The Guinea Pig anti-Rabbit IgG antibody ABIN101961 is well suited as a CUT&RUN IgG negative control and as a secondary antibody in CUT&Tag. It is a component of all our **CUT&RUN Product Sets**. Find more products for CUT&RUN and CUT&Tag: • Overview of **CUT&RUN Antibodies**. • **Magnetic Concanavalin A beads (agarose) for CUT&RUN (ABIN6952467)**. • **CUTANA™ pAG-MNase for ChIC/CUT&RUN Assays (ABIN6950951)**.
Immunogen:	whole molecule of rabbit IgG
Isotype:	IgG
Specificity:	Rabbit IgG (H&L)
Cross-Reactivity (Details):	No reaction was observed against Human, Mouse and Goat Serum Proteins.

Product Details

Purification:	Preadsorption: Solid phase absorption
Sterility:	Sterile filtered

Target Details

Target:	IgG
Abstract:	IgG Products
Target Type:	Antibody

Application Details

Application Notes:	The guinea pig anti-rabbit IgG antibody ABIN101961 is suitable for use in ELISA, immunohistochemistry, and Western Blot, CUT&RUN and CUT&Tag. Specific conditions for each assay should be optimized by the end user. General ABIN101961 dilution recommendations for different applications are as follows: • ELISA: 1:20,000 - 1:100,000 • WB: 1:2,000 - 1:10,000 • IHC: 1:1,000 - 1:5,000 • CUT&RUN: 1:100 • CUT&Tag: 1:100
Comment:	ABIN101961 is tested via ELISA to ensure that the titer against the antigen (Rb IgG) is above a certain threshold. We also test to make sure the titer against potentially cross-reactive human IgG, goat IgG, and mouse IgG is below a certain threshold. In addition, we test ABIN101961 against anti-guinea pig Serum, rabbit IgG, and rabbit serum in an immunoelectrophoresis assay.
Restrictions:	For Research Use only

Handling

Format:	Liquid
Concentration:	1.21 mg/mL
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2, 0.01 % (w/v) NaN3, no stabilizer
Preservative:	Sodium azide
Precaution of Use:	This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.

Handling

Storage:	4 °C,-20 °C
Expiry Date:	12 months

Publications

Product cited in:

Yu, Spiegel, Melidis, Hui, Zhang, Radzevičius, Balasubramanian: "Chem-map profiles drug binding to chromatin in cells." in: **Nature biotechnology**, (2023) (PubMed).

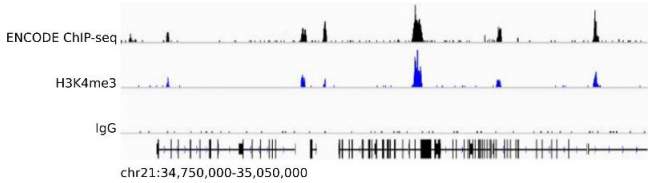
Lu, Ellegast, Ross, Malone, Lin, Mabe, Dharia, Meyer, Conway, Su, Selich-Anderson, Taslim, Byrum, Seong, Adane, Gray, Rivera, Lessnick, Stegmaier: "The ETS transcription factor ETV6 constrains the transcriptional activity of EWS-FLI to promote Ewing sarcoma." in: **Nature cell biology**, Vol. 25, Issue 2, pp. 285-297, (2023) (PubMed).

Zhou, Halstead, Bonnet-Garnier, Schultz, Ross: "Histone remodeling reflects conserved mechanisms of bovine and human preimplantation development." in: **EMBO reports**, Vol. 24, Issue 3, pp. e55726, (2023) (PubMed).

Li, Gao, Zhao, Guan, Morris, Finkelman, Huang: "The Hdc GC box is critical for Hdc gene transcription and histamine-mediated anaphylaxis." in: **The Journal of allergy and clinical immunology**, (2023) (PubMed).

Boileau, Chen, Blelloch: "Loss of MLL3/4 decouples enhancer H3K4 monomethylation, H3K27 acetylation, and gene activation during embryonic stem cell differentiation." in: **Genome biology** , Vol. 24, Issue 1, pp. 41, (2023) (PubMed).

There are more publications referencing this product on: Product page



Cleavage Under Targets and Tagmentation

Image 1. CUT&Tag data produced from human K562 cells using ABIN101961 as a secondary antibody in conjunction with an H3K4me3 antibody (middle) or without a primary antibody as negative control (bottom) in comparison to an ENCODE ChIP-seq data set (top). Adapted from Henikoff et al (2020) eLife, PMID: 33191916

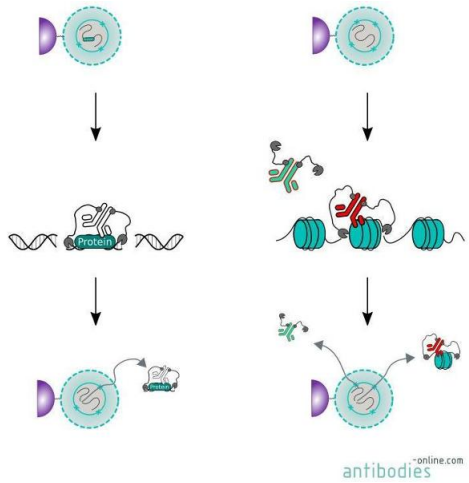


Image 2. Summary of the CUT&RUN protocol (left). Subsequently to immobilization on ConA beads (purple) and permeabilization, cells are incubated with an antibody (white) specific for the protein of interest. Protein A and Protein G-MNase fusion protein (ABIN6950951, grey) is then tethered to the antibody's Fc region and the MNase cleaves the DNA under the target protein. Cleavage products diffuse ouf of the cell and can be further processed for sequencing. As positive control (ABIN6923144, red) and negative control ABIN101961 (turquois) serve antibodies that either bind to an abundant protein or that do not bind to any antigen in the cell (right).

antibodies-online™ Sets				R	M	
CUT&RUN Pro Complete ABIN6923135	✓	✓	✓		✓	✓
CUT&RUN Pro Direct ABIN6923136	✓	✓		✓		✓
CUT&RUN Pro Sec ABIN6923137	✓	✓	✓			✓
CUT&RUN Pro ABIN6923138	✓	✓				✓
CUT&RUN Core Complete ABIN6923131	✓	✓	✓		✓	
CUT&RUN Core Direct ABIN6923132	✓	✓		✓		
CUT&RUN Core Sec ABIN6923133	✓	✓	✓			
CUT&RUN Core ABIN6923134	✓	✓				

Image 3. antibodies-online CUT&RUN sets including the guinea pig anti-rabbit secondary antibody ABIN101961.

Successfully validated (Cleavage Under Targets and Tagmentation (CUT&Tag))

by Tom Taghon's lab, Vakgroep Diagnostische Wetenschappen, Universiteit Gent

Report Number: 104174

Date: Dec 18 2019

Target:	rabbit IgG
Lot Number:	43586
Method validated:	Cleavage Under Targets and Tagmentation (CUT&Tag)
Positive Control:	rabbit anti-H3K27me3 monoclonal antibody
Negative Control:	rabbit normal IgG antibody
Notes:	Passed. ABIN101961 successfully increased the number of protein A binding sites in a CUT&Tag protocol on human primary thymocytes and PER-117 cells using a monoclonal rabbit H3K27me3 primary antibody.
Primary Antibody:	rabbit IgG anti-H3K27me3 antibody (Cell Signaling Technology, 9733T, lot 14)
Secondary Antibody:	ABIN101961

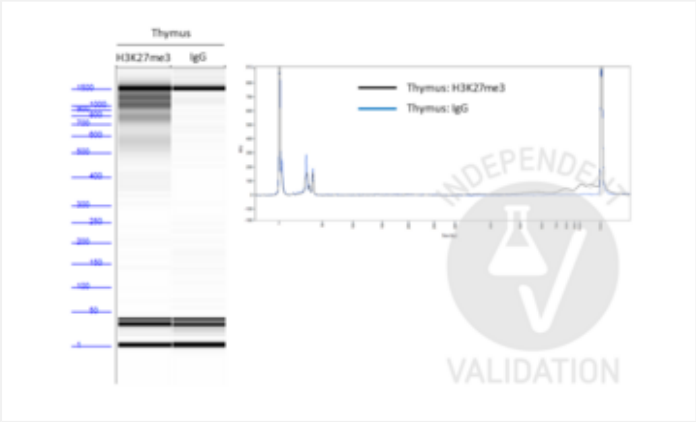
Protocol:	• Profiling of H3K27me3 signals in human primary CD34+ sorted thymocytes and PER-117 cell line (50,000 cells each).
	• Carry out the CUT&Tag protocol according to the single-tube bench top protocol for CUT&Tag developed by Steven Henikoff's lab as outlined on the protocols.io platform.
	• Use reagents as suggested in the original protocol. The pA-Tn5 fusion protein used in this validation was a courtesy of Steven Henikoff's lab and used at 1:1000 dilution for 100 µL per sample.
	• Primary antibody binding with
	  ○ 1µl/sample monoclonal rabbit IgG anti-H3K27me3 antibody (Cell Signaling Technology, 9733T, lot 14) or
	  ○ 1µl/sample rabbit normal IgG (Cell Signaling, 2729S, lot 9).
	• Secondary antibody binding with 1µl/sample guinea pig anti rabbit antibody (antibodies-online, ABIN101961, lot 43586).
	• Determine library concentration on a Qubit Fluorometer using Qubit dsDNA High Sensitivity Assay Kit (ThermoFisher Scientific, Q32581).
	• Determine library size distribution of all samples on an Agilent Fragment Analyzer using High Sensitivity Small DNA Fragment Analysis Kit (Agilent, DNF-477-0500).

Experimental Notes:	• ABIN101961 successfully increased the number of protein A binding sites for each bound

rabbit anti-H3K27me3 antibody in the human primary thymocytes and PER-117 cell line. This resulted in quantifiable amounts of tagmented genomic fragments after PCR amplification that showed a ladder-like distribution.

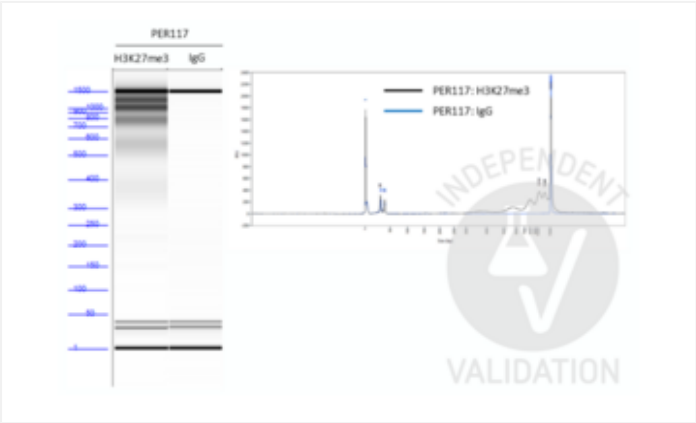
- Library concentrations as measured on a Qubit Fluorometer were 0.122ng/µl and 0.538ng/µl for thymocytes and PER-117 cells respectively using a rabbit H3K27me3 primary antibody. Library concentration for both sample was below the instrument's detections limit using the rabbit normal IgG primary antibody.
- As discussed at the protocols.io, it is expected to observe a ladder-like distribution for H3K27me3 profiling. However, using lower cell numbers could alter the nucleosomal patterns and result in an increase in larger fragments. This is observed by Steven Henikoff's lab as well. Nevertheless, it does not affect the quality of the sequencing results.

Images for Validation report #104174



Validation image no. 1 for Guinea Pig anti-Rabbit IgG (Heavy & Light Chain) antibody - Preadsorbed (ABIN101961)

CUT&Tag library generated from thymocytes using a monoclonal rabbit anti-H3K27me3 primary antibody and ABIN101961 as a secondary antibody.



Validation image no. 2 for Guinea Pig anti-Rabbit IgG (Heavy & Light Chain) antibody - Preadsorbed (ABIN101961)

CUT&Tag library generated from PER-117 cells using a monoclonal rabbit anti-H3K27me3 primary antibody and ABIN101961 as a secondary antibody.