

Datasheet for ABIN3181074  
**anti-DYKDDDDK Tag antibody**[Go to Product page](#)

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## Overview

Quantity:	100 µL
Target:	DYKDDDDK Tag
Reactivity:	Please inquire
Host:	Mouse
Clonality:	Monoclonal
Application:	Western Blotting (WB), Immunoprecipitation (IP), Immunofluorescence (IF)

## Product Details

Sequence:	DYKDDDDK
Clone:	2C5
Isotype:	IgG1
Specificity:	The antibody detects C-terminal, internal, and N-terminal Flag-tag fusion proteins.
Characteristics:	Mouse Monoclonal to Flag-Tag.
Purification:	The antibody was affinity-purified from mouse ascites by affinity-chromatography using specific immunogen.

## Target Details

Target:	DYKDDDDK Tag
Alternative Name:	Flag-Tag ( <a href="#">DYKDDDDK Tag Products</a> )
Target Type:	Tag

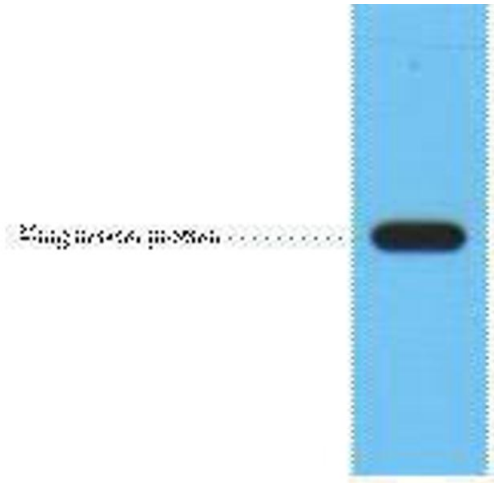
## Application Details

Application Notes:	WB 1:5000, IP 1:200, IF 1:2000
Restrictions:	For Research Use only

## Handling

Format:	Liquid
Concentration:	1 mg/mL
Buffer:	Liquid in PBS containing 50 % glycerol, 0.5 % BSA and 0.02 % sodium azide.
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 Advice:	Avoid repeated freeze/thaw cycles.
Storage:	-20 °C
Storage Comment:	Store at -20°C.

## Images

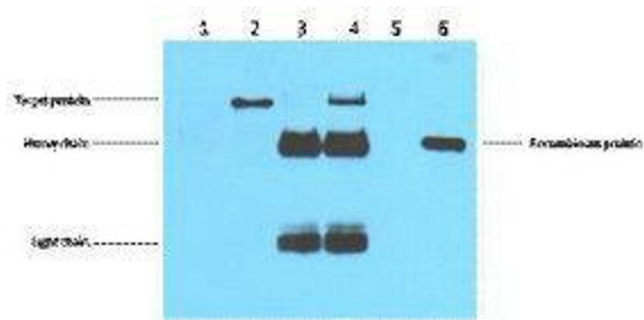


**Western Blotting**

**Image 1.**

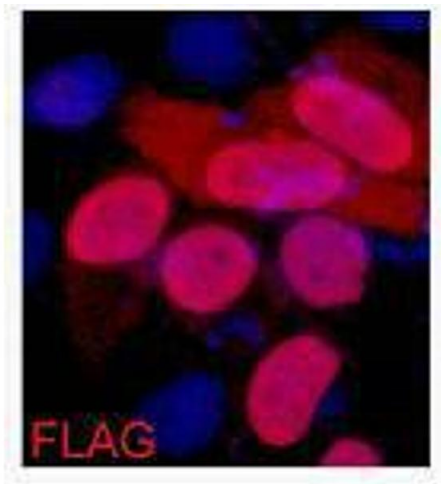
Immunoprecipitation

**Image 2.** Immunoprecipitation (IP) analysis: 5ug Flag Mouse IgG1 per ml Lysate, WB 1:5000.



Immunofluorescence

**Image 3.** Immunofluorescence (IF) analysis of 293 cells transfected with a Flag-tag protein, 1:2000 dilution (blue DAPI, red anti-Flag)





## Successfully validated (Western Blotting (WB))

by [Developmental Biology, Johann-Friedrich-Blumenbach-Institute for Zoology and Anthropology, Georg-August-University of Göttingen](#)

Report Number: 102752

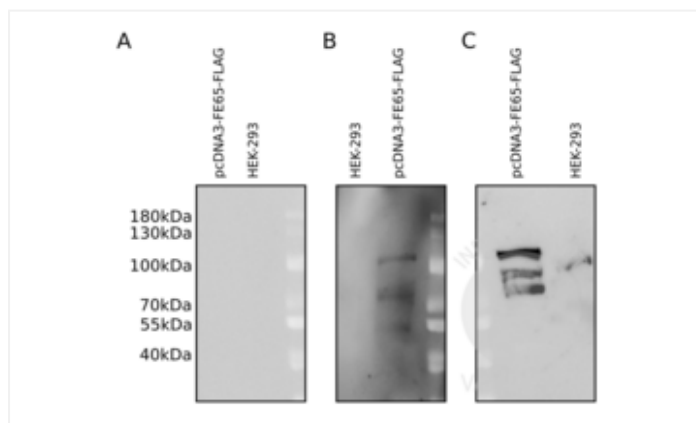
Date: Apr 06 2018

Target:	DYKDDDDK Tag
Lot Number:	02R690396
Method validated:	Western Blotting (WB)
Positive Control:	HEK-293 cells transfected with pcDNA3-FE65-FLAG
Negative Control:	non-transfected HEK-293 cells
Notes:	Passed. ABIN3181074 detects a protein of interest in addition to two further bands.
Primary Antibody:	ABIN3181074
Secondary Antibody:	goat anti-mouse IgG, HRP-linked (Sigma A9044, lot 034M4761)
Protocol:	<ul style="list-style-type: none"> <li>• Grow HEK-293 cells (ATCC, CRL-1573) in DMEM+GlutaMAX (Gibco, 10567-014, lot 1922818) supplemented with fetal bovine serum (Gibco, 10500-064, Lot 08FO477K) and Pen/Strep (Gibco, 15140-122, lot 1924797), at 37°C and 5% CO<sub>2</sub> to 70% confluency.</li> <li>• Transfect cells with Flag-tagged FE65 expression plasmid (pcDNA3-FE65-FLAG; a kind gift of Dr. Sascha Weggen, University of Düsseldorf) using EndofectinMax (GeneCopoeia) following the manufacturer's instructions.</li> <li>• Grow cells for 24h.</li> <li>• Lyse cells in SDS-sample buffer and denature total cellular lysates for 5min at 95°C. Subsequently separate them on a denaturing 10% SDS-PAGE gel (Laemmli 1970) alongside PageRuler Prestained Protein Ladder (ThermoFisher Scientific, 26616).</li> <li>• Transfer proteins onto 0.2µm Protran membrane (GE Healthcare, 10600004, A10043108) with a western blotting system for 1h at 100V (Towbin et al., 1979).</li> <li>• Block the membrane in TBST (50mM Tris-HCl pH7.4, 150mM NaCl, 0.2% Tween 20) containing 5% milk (blocking solution) for 60min at RT.</li> <li>• Incubate membrane with primary mouse anti-DYKDDDDK antibody (antibodies-online, ABIN3181074, lot 02R690396) diluted 1:2000 in blocking solution ON at 4°C.</li> <li>• Wash membrane with TBST for 45min at RT.</li> <li>• Incubate membrane with secondary goat anti-mouse IgG, HRP-linked (Sigma A9044, lot 034M4761) diluted 1:2500 in blocking solution for 45min at RT.</li> <li>• Wash membrane 6x 5min each with TBST.</li> <li>• Reveal protein bands using Clarity Max Western ECL substrate (Bio-Rad, 1705062) and capture images via Chemidoc Imaging System (BioRad).</li> </ul>

- Incubate a secondary blot membrane with primary rabbit anti-Flag tag antibody (Sigma F7425, lot 086m4803V) diluted 1:10000 in blocking solution ON at 4°C.
- Wash membrane with TBST for 45min at RT.
- Incubate membrane with secondary anti-rabbit IgG (H+L), HRP-linked (Jackson ImmunoResearch, 111-035-003, lot 123450) diluted 1:10000 in blocking solution for 45min at RT.
- Wash membrane with TBST for 45min at RT.
- Reveal protein bands using Clarity Max Western ECL substrate (Bio-Rad, 1705062); image capture via Chemidoc Imaging System (BioRad).

Experimental Notes: ABIN3181074 does not detect proteins when diluted 1:5000. It does however detect a protein in the expected molecular mass range at approximately 97kDa as well as two additional proteins when diluted 1:2000. A rabbit anti-Flag tag antibody used as reference detects a protein in the expected molecular mass range as well two additional proteins.

## Image for Validation report #102752



### Validation image no. 1 for anti-DYKDDDDK Tag antibody (ABIN3181074)

Western blot analysis of lysates of HEK-293 cells expressing a DYKDDDDK-tagged protein (pcDNA3-FE65-FLAG) or without any heterologous protein expression (HEK-293). The blot membrane was incubated with ABIN3181074 diluted 1:5000 (A) or 1:2000 (B) or with a rabbit anti-Flag tag antibody diluted 1:10000 (C) followed by secondary antibody incubation and chemiluminescence.



## Successfully validated (Immunocytochemistry (ICC))

by [Developmental Biology, Johann-Friedrich-Blumenbach-Institute for Zoology and Anthropology, Georg-August-University of Göttingen](#)

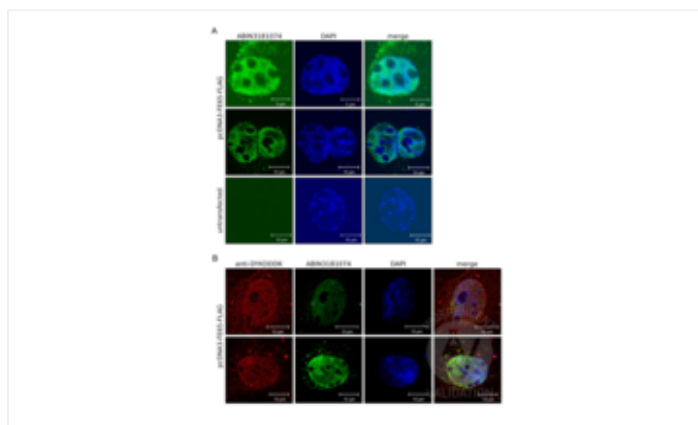
Report Number: 103031

Date: Apr 06 2018

Target:	DYKDDDDK Tag
Lot Number:	02R690396
Method validated:	Immunocytochemistry (ICC)
Positive Control:	NIH/3T3 cells transfected with pcDNA3-FE65-FLAG
Negative Control:	Non-transfected NIH/3T3 cells
Notes:	Passed. ABIN3181074 specifically recognizes DYKDDDDK-labeled protein in ICC.
Primary Antibody:	ABIN3181074
Secondary Antibody:	goat anti-mouse IgG Dylight488 (ThermoFisher Scientific, 35503, lot KA)
Protocol:	<ul style="list-style-type: none"> <li>• Grow NIH/3T3 cells (ATCC, CRL-1658) in DMEM+GlutaMAX (Gibco, 10567-014, Lot 1922818) supplemented with fetal bovine serum (Gibco, 10500-064, lot 08FO477K) and Pen/Strep (Gibco, 15140-122, Lot 1924797), at 37°C and 5% CO<sub>2</sub> to 70% confluency.</li> <li>• Transfect cells with DYKDDDDK-tagged FE65 expression plasmid (pcDNA3-FE65-FLAG; a kind gift of Dr. Sascha Weggen, University of Düsseldorf) using EndofectinMax (GeneCopoeia) following the manufacturer's instructions.</li> <li>• Grow cells for 24h.</li> <li>• Fix cells in 3.7% paraformaldehyde (in PBS) for 20min at 4°C followed by incubation in 0.3% Triton X-100 for 10min at 4°C.</li> <li>• Block cells in PBS containing 1% bovine serum albumin and 0.5% Tween-20 (PBT) for 1h at RT.</li> <li>• Incubate cells with primary <ul style="list-style-type: none"> <li>mouse anti- DYKDDDDK antibody (antibodies-online, ABIN3181074, lot 02R690396) diluted 1:2000 in PBS ON at 4°C or</li> <li>○ rabbit anti-DYKDDDDK tag antibody (Sigma, F7425, Lot 086m4803V) diluted 1:2000 in PBS ON at 4°C.</li> </ul> </li> <li>• Wash cells with TBST (50mM Tris-HCl pH7.4, 150mM NaCl, 0.1% Tween 20) for 15min at RT.</li> <li>• Incubate cells with secondary <ul style="list-style-type: none"> <li>goat anti-mouse IgG Dylight488 (ThermoFisher Scientific, 35503, lot KA) diluted 1:500 in PBS for 1h at 37°C or</li> <li>○ goat anti-rabbit IgG MFP590 (Mabtec, MFP-A1037, lot 3002088) diluted 1:500 in PBS for 1h at 37°C.</li> </ul> </li> </ul>

- Counterstain DNA with DAPI (Sigma, D9542).
- Image acquisition on Zeiss LSM 510 confocal microscope and processing using Adobe Photoshop 5.0.

## Image for Validation report #103031



### Validation image no. 1 for anti-DYKDDDDK Tag antibody (ABIN3181074)

A ABIN3181074 (green) detects a heterologously expressed DYKDDDDK-tagged protein in HEK-293 cells (pcDNA3-FE65-FLAG) but does not reveal an antigen in untransfected cells (untransfected). The pictures on the right show the green channel merged with DAPI counterstain (blue). B A different anti-DYKDDDDK tag antibody (red) was used as control for ABIN3181074 (green). The pictures on the right show the red and green channels merged with DAPI counterstain (blue).