Technical Info

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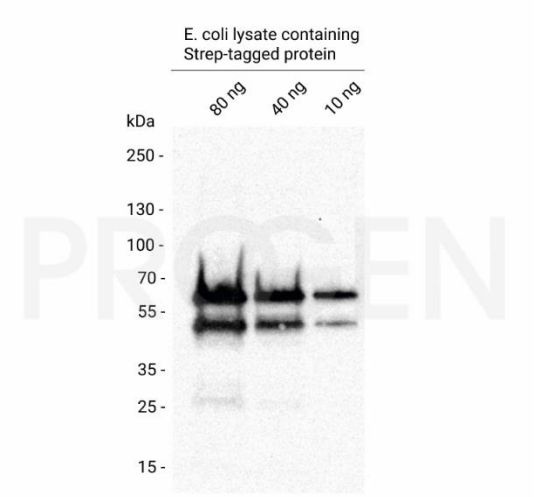


PROGEN Antibodies

General information

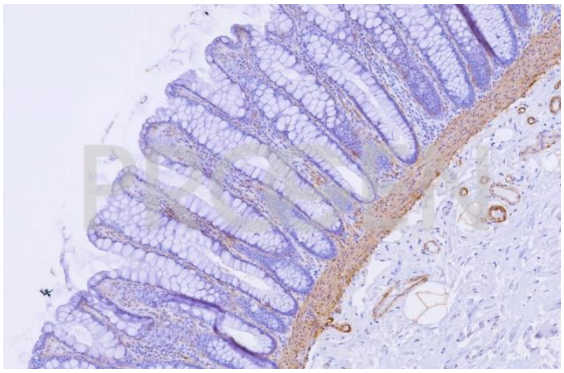
Our antibody assortment from PROGEN contains various monoclonal primary antibodies, which are used in a variety of different research areas. The antibodies have a high epitope affinity and are excellently suited for cell and tissue biology studies, or also for protein biochemical analyses.

PROGEN was founded in 1983 and has been DIN ISO 13485 certified since 2010. High quality is guaranteed through cooperation with leading research groups in the production of antibodies.

Applications

Our range of PROGEN includes various primary monoclonal antibodies, obtained from mice, for the detection of protein tags, or for the detection of specific cell or tissue components. The following applications can be performed with the antibodies (for more detailed information, please see our overview on p.2):

- Dot Blots
- Enzyme-linked Immunosorbent Assays (**ELISA**)
- Immunohistochemistry (**IHC**)
- Immunoprecipitation (**IP**)
- Immunocytochemistry/Immunofluorescence (**ICC/IF**)
- Western Blots (**WB**)



The high quality of PROGEN antibodies is also assured by various control tests.
The quality of each **IHC**, **ICC** and **IF** antibody lot is controlled externally by testing on relevant tissue sections or cells.
The evaluation of antibody performance in **WB** is ensured by using specific WB positive controls.
To characterise the selectivity and specificity of the antibodies, they are validated by epitope mapping and tested for cross reactivity.

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Overview

Marker-Cat.	Antibody	Applications	Reactivity	Art. No.
Tag-Antibody	anti-6-His-tag mouse monoclonal, 6His	ICC/IF, IP, WB	6-His-tag	1NNA
Tag-Antibody	anti-c-myc-tag mouse monoclonal, 9E10	IP, WB	c-myc-tag	1NNT
Tag-Antibody	anti-DDDDK-tag mouse monoclonal, AP1501	ICC/IF, IP, WB	DDDDK-tag	1NLE
Tag-Antibody	anti-GFP-tag mouse monoclonal, F56-6A1.2.3	ICC/IF, WB	GFP-tag	1NP5
Tag-Antibody	anti-GST-tag mouse monoclonal, F50-3D12.2	WB	GST-tag	1NN8
Tag-Antibody	anti-HA-tag mouse monoclonal, 12CA5	WB	HA-tag	1NN7
Tag-Antibody	anti-Strep-tag mouse monoclonal, C23.21	WB	Strep-tag	1NLL
Tag/Cell Membrane	anti-Alkaline Phosphatase (intestinal) mouse monoclonal, V17.1	IHC, ELISA	bovine, human	1NLX
Cell-cell contact	anti-Desmocollin 1 mouse monoclonal, Dsc1-U100	IHC, WB	human, mouse, rat	1NN6
Cell-cell contact	anti-Desmocollin 3 mouse monoclonal, Dsc3-U114	IHC, WB	human, mouse, rat	1NNL
Cell-cell contact	anti-Desmoglein 1/2 mouse monoclonal, DG 3.10	ICC/IF, IHC, WB	bovine, human, rat	1NP0
Cell-cell contact	anti-Desmoglein 2 mouse monoclonal, 10G11	IHC, WB	human	1NLT
Cell-cell contact	anti-Plakoglobin mouse monoclonal, PG 5.1.	ICC/IF, IHC, WB	bovine, chicken, human, mouse, rat, zebrafish	1NNC
Cell cycle	anti-Cyclin D1 mouse monoclonal, DCS-6,	ICC/IF, IHC, WB	dog, human, monkey, mouse, rat	1NNX
Cell cycle	anti-Cyclin-Dependent Kinase 4 mouse monoclonal, DCS-156	WB	human, mouse, rat	1NNE
Cell cycle	anti-p16 Protein mouse monoclonal, DCS-50	ICC/IF, IHC, WB	human	1NN9
Cell cycle	anti-p53 Protein mouse monoclonal, Bp53.11	ICC/IF, IHC, WB	human	1NNY
Cytoskeleton	anti-alpha-Smooth Muscle Actin mouse monoclonal, 1A4/ ASM-1	ICC/IF, IHC, WB	bovine, chicken, equine, human, mouse, rat	1NN1
Cytoskeleton	anti-Cardiac Actin mouse monoclonal, AC1-20.4.2,	IHC, WB	bovine, chicken, human, rabbit	1NLN
Cytoskeleton	anti-Vimentin mouse monoclonal, VIM 3B4	ICC/IF, IHC, WB	amphibia, bovine, chicken, human, monkey	1NP3
Cytoplasm/ Cytoskeleton	anti-beta-actin, clone AC-15, mouse monoclonal	WB, IHC	pig, bovine, rat, chicken, human, rabbit, mouse, guinea pig, canine	1NP6
Cytoplasm Podocytes/Cerebrum	anti-Synaptopodin/SYNPO mouse monoclonal, G1D4	ICC/IF, IHC, WB	human, mouse, rat (not with rabbit, frog, chicken)	1NP2
Epithelial marker	anti-Desmoplakin 1/2 mouse monoclonal, DP 1 + 2-2.15,	ICC/IF, IHC, WB	bovine, chicken, human, mouse, rat	1NP7
Epithelial marker	anti-EP-CAM mouse monoclonal, HEA125, liquid, purified	ICC/IF, IHC, WB	human (negative with mouse)	1NP1
Epithelial marker	anti-Keratin K17 mouse monoclonal, Ks17.E3	IHC, WB	human, rat	1NN2
Epithelial marker	anti-Keratin K18 mouse monoclonal, Ks18.04	ICC/IF, IHC, WB	bovine, dog, hamster, human, mouse, pig, rat, sheep, trout, zebrafish	1NLY
Epithelial marker	anti-Keratin K19 mouse monoclonal, Ks19.2 (Z105.6)	ICC/IF, IHC, WB, ELISA	bovine, human, rabbit, rat (negative with mouse, woodchuck, chicken, xenopus)	1NNK
Epithelial marker	anti-Keratin K20 mouse monoclonal, IT-Ks20.8	IHC, WB	human, mouse	1NNP
Epithelial marker	anti-Keratin K5/K8 (Pan Epithelial) mouse monoclonal, C22	ICC/IF, IHC, WB	amphibia, bovine, human, hydra, mouse, pig	1NN0
Epithelial marker	anti-Keratin K6 mouse monoclonal, KA12	IHC, WB	human, mouse, rat	1NN4
Epithelial marker	anti-Keratin K7 mouse monoclonal, Ks7.18	ICC/IF, IHC, WB	bovine, human, pig, sheep	1NNN
Epithelial marker	anti-Keratin Type II mouse monoclonal, Ks pan1-8, liquid, purified	ICC/IF, IHC, WB	bovine, chicken, human, mouse, Pleurodelis, rat, snake, xenopus	1NNH
Epithelial marker /Cell Membrane	anti-Uroplakin III mouse monoclonal, AU1, liquid, purified	IHC, WB	bovine, human, pig, rat	1NLK
Glial cell marker	anti-Glial Fibrillary Acidic Protein mouse monoclonal, GF 12.24	ICC/IF, IHC, WB	bovine, human, mouse, rat	1NP8
Nucleus	anti-DNA mouse monoclonal, AC-30-10, liquid, purified	ICC/IF, IHC, Dot Blot	all species	1NN5
Lipid marker	anti-Perilipin 1 (N-terminus) mouse monoclonal, PERI 112.17	ICC/IF, IHC, WB	bovine, human, rat	1NLH
Lipid marker	anti-Perilipin 2 (N-terminus) mouse monoclonal, AP125	IHC, WB	dog, human, rat	1NP4
Loading control	anti-GAPDH, clone 6C5	WB	human, mouse, rat, rabbit, fish, pig	1NLP
Tumour marker neuronal/adrenal	anti-Synaptophysin mouse monoclonal, SY38	ICC/IF, IHC, WB	bovine, human, mouse, rat	1NN3

Technical Info

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Protocols and product proposals

The following section contains PROGEN protocol suggestions for Western blot and immunohistochemistry with which the antibodies have already been successfully tested.

The recommended products from our range have not been tested specifically but are very suitable for these biochemical analyses.

Product proposals for the use of antibodies according to the PROGEN protocol

Product used	Recommended product	Art. No.
RIPA lysis buffer		
• Tris-HCl	TRIS hydrochloride, PUFFERAN® ≥99 %, p.a.	9090.1
• NaCl	Sodium chloride, CELLPURE® ≥99.5 %	HN00.1
• NP-40	Tergitol™ 15-S-9, extra pure (final conc. 1.2%)	9975.1
• Sodium Deoxycholat	Deoxycholic acid sodium salt, ≥98 %, for biochemistry	3484.1
• SDS	ROTI®Stock 20% SDS	1057.1
• Protease inhibitors	Inhibitor Cocktail Standard, for biochemistry	3743.1
NP-40 buffer		
• Tris-HCl	TRIS hydrochloride, PUFFERAN® ≥99 %, p.a.	9090.1
• NaCl	Sodium chloride, CELLPURE® ≥99,5 %	HN00.1
• NP-40	Tergitol™ 15-S-9, extra pure (final conc. 1.2%)	9975.1
• Protease inhibitors	Inhibitor Cocktail Standard, for biochemistry	3743.1
3x Laemmli sample buffer	ROTI®Load 1, 4x konz., reduzierend	K929.1
10x PBS	ROTI®Stock 10x PBS, BioScience Grade, sterile filtered	1058.1
	ROTI®Fair PBS 7.4, for 1000 ml/tablet, for biochemistry	1112.1
Blocking buffer		
• Non-fat dry milk	Powdered milk, Blotting Grade, powdered, low in fat	T145.1
	ROTI®Block, 10x conc. *ready-to-use*	A151.1
• PBST	ROTI®Stock 10x PBST, BioScience Grade, sterile filtered	1059.1
	ROTI®Fair PBST 7.4, for 1000 ml/tablet, for biochemistry	1116.1
	ROTI®PreMix PBST, for biochemistry and molecular biology	0987.1
10x Citrat buffer		
• tri-Natriumcitrat-Dihydrat	tri-Sodium citrate dihydrate, CELLPURE® ≥99 %	HN12.1
• Zitronensäure	Citric acid, ≥99,5 %, p.a., ACS, anhydrous	X863.1
10x PBS	ROTI®Stock 10x PBS, BioScience Grade, sterile filtered	1058.1
	ROTI®Fair PBS 7.4, for 1000 ml/tablet, for biochemistry	1112.1
Xylol	Xylene (isomers), ≥97 %, pure, for histology	9713.1
Ethanol	Ethanol, ≥99.8%, for molecular biology	1HPH.1
DAKO-Pen	ROTI®Liquid Barrier Marker, colourless, for microscopy	AN91.1
Normal serum	ROTI®ImmunoBlock, 10x conc.	T144.1
DAB solution	ROTI®DAB Kit, for immunochemistry	9202.1
Haemalaun	Hemalum solution acid acc. to Mayer, for microscopy	T865.1
Eukitt	ROTI®Histokitt II, for histology	T160.1

➢ Further solutions, reagents or devices can be found in our webshop.

Western Blot Protocol

Solutions and reagents

Lysis buffers

RIPA buffer (radioimmunoprecipitation assay buffer):

- 50 mM Tris-HCl, pH 8.0
- 150 mM NaCl
- 1% NP-40 or 0.1% Triton X-100
- 0.5% sodium deoxycholate
- 0.1% SDS
- protease inhibitors

NP-40 buffer:

- 50 mM Tris-HCl, pH 8.0
- 150 mM NaCl
- 1.0% NP-40 (or 0.1% Triton X-100)
- protease inhibitors

3 x Laemmli buffer/ sample buffer:

- 150 mM Tris-HCl, pH 6.8
- 300 mM DTT
- 6% SDS
- 0.3% bromophenol blue
- 30% glycerol

10 x PBS:

- 1.37 M NaCl
- 0.027 M KCl
- 0.1 M Na2HPO4
- 0.018 M KH2PO4

1. Resolve in 800 ml ddH2O.
2. Adjust pH to 7.4 using HCl.
3. Fill it up to 1l.
4. Autoclave it and store at room temperature.
5. Dilute 1:10 before use.

blocking buffer:

- 3–5% non-fat dry milk or BSA
- in PBST (PBS + 0.1% Tween 20)

Procedure

Sample preparation – lysate from cell culture

1. Place the cell culture dish on ice and wash the cells with ice-cold PBS.
2. Aspirate the PBS and add ice-cold lysis buffer (1 ml per 10 cm dish).
3. Scrape adherent cells off the dish using a cold plastic cell scraper and gently transfer the cell suspension into a precooled microcentrifuge tube.
4. If required, cells can be harvested by trypsinization and washed with PBS prior to resuspension in lysis buffer.
5. Incubate at 4°C for 30 min with constant agitation, centrifuge at 16,000 x g for 20 min at 4°C.
6. Transfer the supernatant to a fresh tube on ice, and discard the pellet.
7. Remove a small volume (10-20 µl) of lysate for analysis by a protein assay. Determine the protein concentration for each cell lysate.
8. If necessary, aliquot the protein samples for long-term storage at -20°C. Repeated freeze and thaw cycles cause protein degradation and should be avoided.
9. Add ½ volume of 3x Laemmli sample buffer.
10. Boil each cell lysate in sample buffer at 95°C for 5 min.
11. Centrifuge at 16,000 x g in a microcentrifuge for 1 min.

Protein separation by SDS-PAGE

Polyacrylamid percentage of SDS-gel for best resolution of proteins based on their molecular weight:

Protein size	Gel percentage
4 – 40 kDa	20%
12 – 45 kDa	15%
10 – 70 kDa	12,5
15 – 100 kDa	10%
25 – 200 kDa	8%

1. Load equal amounts of protein into the wells of the SDS-PAGE (10 – 50 µg/lane protein of cell lysate or 10 – 100 ng/lane purified protein). Add molecular weight marker in one of the lanes.
2. Run the gel according to manufacturer's instructions (e.g. 1 – 2 h at 200 V).

Protein transfer from gel to membrane

Use either nitrocellulose or PVDF membrane. Activate PVDF with methanol for 1 min and rinse with transfer buffer before preparing the stack. Follow the manufacturer's instructions for blotting.

Antibody incubation

1. After transfer, briefly rinse the membrane in distilled water or PBST.
2. Block the membrane 1 h at RT with blocking buffer.
3. Incubate the membrane with appropriate dilutions of primary antibody in blocking buffer ON at 4°C or 1-2 h at RT.
4. Wash the membrane with PBST for 15 min/ 3x 5 min.
5. Incubate the membrane with recommended dilution of conjugated secondary antibody in blocking buffer for 1 h at RT.
6. Wash the membrane with PBST for 15 min/ 3x 5 min.
7. For signal development follow the kit manufactures instructions of the detection kit used.

IHC Protocol (paraffin)
Mouse primary antibody

Solutions and reagents

Lysis buffers

10 x Citrate Buffer:

- 29.4 g Tris-Sodium Citrate 2-hydrate ($C_6H_5Na_3O_72H_2O$)(= 0.1 M)

1. Resolve in 800 ml ddH_2O.
2. Adjust pH to 6.0 using citric acid.
3. Fill it up to 1 l.
4. Store at 4°C.
5. Dilute 1:10 before use.

10 x PBS:

- 80 g NaCl
- 2 g KCl
- 14 g Na_2HPO_4
- 2.4 g $KH_2PO_4$15O mM NaCl
- 1.0% NP-40 (or 0.1% Triton X-100)
- protease inhibitors

1. Resolve in 800 ml ddH_2O.
2. Adjust pH to 7.4 using HCl.
3. Fill it up to 1 l.
4. Autoclave it.
5. Store at RT.
6. Dilute 1:10 before use.

Procedure

Deparaffinization

- Xylol 5 min
- Xylol 5 min
- 100% EtOH 3 min
- 100% EtOH 3min
- 3% H_2O_2 in 70% EtOH 10 min
- ddH_2O 1min

1. Antigen retrieval for 30 min in 10 mM Citrate buffer (preheated; start the steam cooker 10 min in advance; use plastic cuvettes for the slides).
2. Let the slides cool down in the buffer to RT (for approximately 40 min).
3. Wash the slides 2x 5 min in 1x PBS (shaking).
4. Encircle the sections with DAKO-Pen during the washing time in 1x PBS.
5. Prepare a moist chamber for the staining procedure.

Staining

1. Incubate the sections with normal serum (VectorLaboratories, MP-7402: ImmPRESS® HRP Anti-Mouse IgG (Peroxidase) Polymer Detection Kit) for 20 min at RT.
2. Remove the normal serum from the sections (knocking off, do not wash!).
3. Dilute the primary antibody with 1x PBS and apply it to the sections.
4. Incubate the primary antibody ON at 4°C or 60 min at RT in a wet chamber.
5. Remove the primary antibody from the slides (knocking off) and wash 2x 5 min in 1x PBS (shaking).
6. Incubate the sections with the secondary antibody (VectorLaboratories, MP-7402: ImmPRESS® HRP Anti-Mouse IgG (Peroxidase) Polymer Detection Kit) for 20 min at RT in a wet chamber.
7. Wash the slides 2x 5 min in 1x PBS (shaking).
8. Prepare the DAB solution (VectorLaborarories, SK-4100) according to manufacturer's recommendations briefly before use and mix it well.
9. Incubate slides with DAB solution until a brown staining is visible. The development time is varying! (from a few seconds to a few minutes; watch closely)
10. Put the slides 3 min in 50 mM $NaHCO_3$.
11. Wash the slides briefly in ddH_2O.
12. Put the slides in Haemalaun (the time is varying-from a few seconds to a few minutes).
13. Wash the slides under rinsing tap water for 10 min.

Alcohol series and Xylol

- 70% EtOH briefly
- 96% EtOH briefly
- 100% EtOH briefly
- 100% EtOH 2 min
- Xylol briefly
- Xylol 2 min

Cover the sections with Eukitt and cover slip.