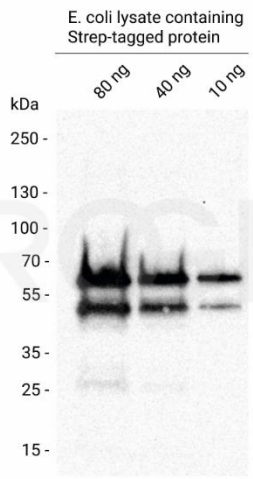
Technische Info

Guter Rat ist Roth.



Antikörper von PROGEN

Allgemeine Informationen

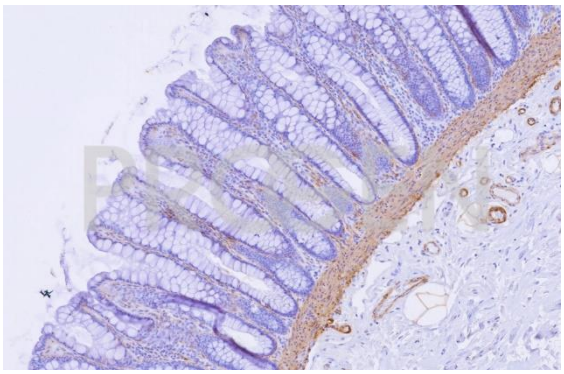
Unser Antikörper Sortiment von der Firma PROGEN beinhaltet verschiedenste monoklonale **Primärantikörper**, welche Anwendung in den unterschiedlichsten Forschungsfeldern finden. Die Antikörper haben eine hohe Epitopaffinität und eignen sich hervorragend für zell- und gewebebiologische Studien, oder auch für proteinbiochemische Analysen.

Die Firma PROGEN wurde im Jahr 1983 gegründet und ist seit dem Jahr 2010 DIN ISO 13485 zertifiziert. Durch die Kooperation mit führenden Forschungsgruppen bei der Herstellung der Antikörper, wird eine hohe Qualität gewährleistet.

Applikationen

Unser Sortiment von PROGEN umfasst verschiedene monoklonale Primärantikörper, gewonnen aus der Maus, für den Nachweis von Protein-Tags, oder zum Nachweis spezifischer Zell- oder Gewebebestandteile. Folgende Applikationen können mit den Antikörpern durchgeführt werden (für genauere Informationen, schauen Sie bitte in unsere Übersicht auf S.2):

- Dot Blots
- Enzyme-linked Immunosorbent Assays (**ELISA**)
- Immunhistochemie (**IHC**)
- Immunopräzipitation (**IP**)
- Immunzytochemie/Immunofluoreszenz (**ICC/IF**)
- Western Blots (**WB**)



Die hohe Qualität der PROGEN-Antikörper wird unter anderem auch durch verschiedene Kontrolluntersuchungen gewährleistet.

Die Qualität jeder **IHC-**, **ICC-** und **IF**-Antikörpercharge wird extern kontrolliert, durch Testung an relevanten Gewebeschnitten, oder Zellen.

Die Verwendung von Antikörpern im **WB** wird gewährleistet, durch die Untersuchung im WB unter Verwendung von spezifischen WB-Positivkontrollen.

Zur Charakterisierung der Selektivität und Spezifität der Antikörper, werden diese durch Epitopkartierung validiert und auf Kreuzreaktivität überprüft.

Übersicht

Marker-Kat.	Antikörper	Applikationen	Reaktivität	Best. Nr.
Tag-Antikörper	anti-6-His-tag mouse monoclonal, 6His	ICC/IF, IP, WB	6-His-tag	1NNA
Tag-Antikörper	anti-c-myc-tag mouse monoclonal, 9E10	IP, WB	c-myc-tag	1NNT
Tag-Antikörper	anti-DDDDK-tag mouse monoclonal, AP1501	ICC/IF, IP, WB	DDDDK-tag	1NLE
Tag-Antikörper	anti-GFP-tag mouse monoclonal, F56-6A1.2.3	ICC/IF, WB	GFP-tag	1NP5
Tag-Antikörper	anti-GST-tag mouse monoclonal, F50-3D12.2	WB	GST-tag	1NN8
Tag-Antikörper	anti-HA-tag mouse monoclonal, 12CA5	WB	HA-tag	1NN7
Tag-Antikörper	anti-Strep-tag mouse monoclonal, C23.21	WB	Strep-tag	1NLL
Tag/Zellmembran	anti-Alkaline Phosphatase (intestinal) mouse monoclonal, V17.1	IHC, ELISA	bovine, human	1NLX
Cytoskelett	anti-alpha-Smooth Muscle Actin mouse monoclonal, 1A4/ ASM-1	ICC/IF, IHC, WB	bovine, chicken, equine, human, mouse, rat	1NN1
Cytoskelett	anti-Cardiac Actin mouse monoclonal, AC1-20.4.2,	IHC, WB	bovine, chicken, human, rabbit	1NLN
Cytoskelett	anti-Vimentin mouse monoclonal, VIM 3B4	ICC/IF, IHC, WB	amphibia, bovine, chicken, human, monkey	1NP3
Cytoplasma/ Cytoskelett	anti-beta-actin, clone AC-15, mouse monoclonal	WB, IHC	pig, bovine, rat, chicken, human, rabbit, mouse, guinea pig, canine	1NP6
Cytoplasma Podozyten/Großhirn	anti-Synaptopodin/SYNPO mouse monoclonal, G1D4	ICC/IF, IHC, WB	human, mouse, rat (not with rabbit, frog, chicken)	1NP2
Epithelmarker	anti-Desmoplakin 1/2 mouse monoclonal, DP 1 + 2-2.15,	ICC/IF, IHC, WB	bovine, chicken, human, mouse, rat	1NP7
Epithelmarker	anti-EP-CAM mouse monoclonal, HEA125, liquid, purified	ICC/IF, IHC, WB	human (negative with mouse)	1NP1
Epithelmarker	anti-Keratin K17 mouse monoclonal, Ks17.E3	IHC, WB	human, rat	1NN2
Epithelmarker	anti-Keratin K18 mouse monoclonal, Ks18.04	ICC/IF, IHC, WB	bovine, dog, hamster, human, mouse, pig, rat, sheep, trout, zebrafish	1NLY
Epithelmarker	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
Epithelmarker	anti-Keratin K20 mouse monoclonal, IT-Ks20.8	IHC, WB	human, mouse	1NNP
Epithelmarker	anti-Keratin K5/K8 (Pan Epithelial) mouse monoclonal, C22	ICC/IF, IHC, WB	amphibia, bovine, human, hydra, mouse, pig	1NN0
Epithelmarker	anti-Keratin K6 mouse monoclonal, KA12	IHC, WB	human, mouse, rat	1NN4
Epithelmarker	anti-Keratin K7 mouse monoclonal, Ks7.18	ICC/IF, IHC, WB	bovine, human, pig, sheep	1NNN
Epithelmarker	anti-Keratin Type II mouse monoclonal, Ks pan1-8, liquid, purified	ICC/IF, IHC, WB	bovine, chicken, human, mouse, Pleurodelis, rat, snake, xenopus	1NNH
Epithelmarker/ Zellmembran	anti-Uroplakin III mouse monoclonal, AU1, liquid, purified	IHC, WB	bovine, human, pig, rat	1NLK
Gliazellenmarker	anti-Glial Fibrillary Acidic Protein mouse monoclonal, GF 12.24	ICC/IF, IHC, WB	bovine, human, mouse, rat	1NP8
Ladekontrolle	anti-GAPDH, clone 6C5	WB	human, mouse, rat, rabbit, fish, pig	1NLP
Lipidmarker	anti-Perilipin 1 (N-terminus) mouse monoclonal, PERI 112.17	ICC/IF, IHC, WB	bovine, human, rat	1NLH
Lipidmarker	anti-Perilipin 2 (N-terminus) mouse monoclonal, AP125	IHC, WB	dog, human, rat	1NP4
Tumormarker neuronal/adrenal	anti-Synaptophysin mouse monoclonal, SY38	ICC/IF, IHC, WB	bovine, human, mouse, rat	1NN3
Zell-Zell Kontakt	anti-Desmocollin 1 mouse monoclonal, Dsc1-U100	IHC, WB	human, mouse, rat	1NN6
Zell-Zell Kontakt	anti-Desmocollin 3 mouse monoclonal, Dsc3-U114	IHC, WB	human, mouse, rat	1NNL
Zell-Zell Kontakt	anti-Desmoglein 1/2 mouse monoclonal, DG 3.10	ICC/IF, IHC, WB	bovine, human, rat	1NP0
Zell-Zell Kontakt	anti-Desmoglein 2 mouse monoclonal, 10G11	IHC, WB	human	1NLT
Zell-Zell Kontakt	anti-Plakoglobin mouse monoclonal, PG 5.1.	ICC/IF, IHC, WB	bovine, chicken, human, mouse, rat, zebrafish	1NNC
Zellkern	anti-DNA mouse monoclonal, AC-30-10, liquid, purified	ICC/IF, IHC, Dot Blot	all species	1NN5
Zellzyklus	anti-Cyclin D1 mouse monoclonal, DCS-6,	ICC/IF, IHC, WB	dog, human, monkey, mouse, rat	1NNX
Zellzyklus	anti-Cyclin-Dependent Kinase 4 mouse monoclonal, DCS-156	WB	human, mouse, rat	1NNE
Zellzyklus	anti-p16 Protein mouse monoclonal, DCS-50	ICC/IF, IHC, WB	human	1NN9
Zellzyklus	anti-p53 Protein mouse monoclonal, Bp53.11	ICC/IF, IHC, WB	human	1NNY

Technische Info

Guter Rat ist Roth.

Protokolle und Produktvorschläge

Im folgenden Abschnitt befinden sich Protokollvorschläge von PROGEN für den **Western-Blot** und für die **Immunhistochemie**, mit welchen die Antikörper bereits erfolgreich getestet wurden.

Die empfohlenen Produkte aus unserem Sortiment wurden nicht im spezifischen getestet, eignen sich aber hervorragend für die biochemischen Analysen.

Produktvorschläge für die Nutzung der Antikörper nach dem PROGEN Protokoll

Verwendetes Produkt	Empfohlenes Sortimentsprodukt	Best. Nr.
RIPA Lyse Puffer		
• Tris-HCl	TRIS Hydrochlorid, PUFFERAN® ≥99 %, p.a.	9090.1
• NaCl	Natriumchlorid, CELLPURE® ≥99,5 %	HN00.1
• NP-40	Tergitol™ 15-S-9, reinst (End.-Konz. 1,2 %)	9975.1
• Natriumdesoxycholat	Desoxycholsäure Natriumsalz, ≥98 %, für die Biochemie	3484.1
• SDS	ROTI®Stock 20 % SDS	1057.1
• Protease Inhibitoren	Inhibitorcocktail Standard, für die Biochemie	3743.1
NP-40 Puffer		
• Tris-HCl	TRIS Hydrochlorid, PUFFERAN® ≥99 %, p.a.	9090.1
• NaCl	Natriumchlorid, CELLPURE® ≥99,5 %	HN00.1
• NP-40	Tergitol™ 15-S-9, reinst (End.-Konz. 1,2 %)	9975.1
• Protease Inhibitoren	Inhibitorcocktail Standard, für die Biochemie	3743.1
3x Laemmli Ladepuffer	ROTI®Load 1, 4x konz., reduzierend	K929.1
10x PBS	ROTI®Stock 10x PBS, BioScience Grade, sterilfiltriert	1058.1
	ROTI®Fair PBS 7.4, für 1000 ml/Tablette, für die Biochemie	1112.1
Blocking-Puffer		
• Magermilchpulver	Milchpulver, Blotting Grade, pulv., fettarm	T145.1
	ROTI®Block, 10x konz. *ready-to-use*	A151.1
• PBST	ROTI®Stock 10x PBST, BioScience Grade, sterilfiltriert	1059.1
	ROTI®Fair PBST 7.4, für 1 000 ml/Tablette, für die Biochemie	1116.1
	ROTI®PreMix PBST, für die Biochemie und Molekularbiologie	0987.1
10x Citrat-Puffer		
• tri-Natriumcitrat-Dihydrat	tri-Natriumcitrat Dihydrat, CELLPURE® ≥99 %	HN12.1
• Zitronensäure	Citronensäure, ≥99,5 %, p.a., ACS, wasserfrei	X863.1
10x PBS	ROTI®Stock 10x PBS, BioScience Grade, sterilfiltriert	1058.1
	ROTI®Fair PBS 7.4, für 1000 ml/Tablette, für die Biochemie	1112.1
Xylol	Xylol (Isomere), ≥97 %, rein, für die Histologie	9713.1
Ethanol	Ethanol, ≥99,8 %, für die Molekularbiologie	1HPH.1
DAKO-PEN	ROTI®Liquid Barrier Marker, farblos, für die Mikroskopie	AN91.1
Normal-Serum	ROTI®ImmunoBlock, 10x konz.	T144.1
DAB-Lösung	ROTI®DAB Kit, für die Immunchemie	9202.1
Hämalaun	Hämalaunlösung sauer nach Mayer, für die Mikroskopie	T865.1
Eukitt	ROTI®Histokitt II, für die Histologie	T160.1

➢ Weitere Lösungen, Reagenzien oder Geräte finden Sie in unserem 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.