3rd Edition

Functional Antibodies

Focus: Recombinant Antibodies

Antibodies are highly specific, naturally evolved molecules that recognize and eliminate pathogenic and disease antigens. The typical antibody consists of two antigen-binding fragments (Fabs), which are linked via a flexible region (the hinge) to a constant Fc region. This structure comprises two pairs of polypeptide chains, each pair containing a heavy and a light chain of different sizes. The Fc portion of the Ig serves to bind various effector molecules of the immune system, as well as molecules that determine the biodistribution of the antibody.

Antibodies are produced by: a) Injecting an antigen into mammals (mouse, rat, rabbit, goat, etc). Blood isolated from these animals contains polyclonal antibodies (multiple antibodies that bind to different epitopes of the same antigen), which are purified. b) Hybridoma technology generating monoclonal antibodies (epitope specific). Specific antibody-secreting lymphocytes are isolated from animals and immortalized by fusing them with a cancer cell line.

Monoclonal antibodies are routinely used in biochemistry, molecular biology, medical research and as therapeutic agents. Important advances have been made over the past decade to improve the specificity and efficacy of such antibodies by new engineering technologies, including recombinant antibody technology, such as antibody phage display (see page 8 for more information).

Functional Grade Antibodies (FuncAbs™):

Antibodies displaying an agonist or antagonist activity (functional grade antibodies (FuncAbs[™])) are powerful tools for mimicking or blocking physiological functions *in vitro* and in vivo. Functional grade antibodies are available free of preservatives and tested for low endotoxin content and may be used for activation, neutralizing or blocking studies, both in vitro or in vivo.

Antigen Binding Fab

General Antibody Schematic

Different Types of Antibody Functionality



Activation/Induction Depletion



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Asc | NLRP3 | cleaved Caspase-1 (p20) Antibodies





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Blocking/Neutralizing Functional Antibodies

anti-APRIL (mouse), mAb (rec.) (blocking) (Apry-1-1)

AG-27B-0001 AG-27B-0001PF Preservative Free AG-27B-0001B Biotin

B Biotin

 Isotype
 Mouse IgG2bλ

 Application
 ELISA, IP, FUNC (Blocking)

Functional Application Inhibits binding of mouse APRIL to mouse BCMA and TACI.

LIT: Production of the plasma-cell survival factor APRIL peaks in myeloid precursor cells from human bone marrow: T. Matthes, et al.; Blood **118**, 1838 (2011)



Prod. No. AG-27B-0017

Newly released: anti-APRIL (mouse), mAb (rec.) (blocking) (Apry-1-3)



anti-Angiopoietin-2 mAb (rec.) (blocking) (Angy-2-1)

AG-27B-0016 AG-27B-0016PF

Preservative Free

100 μg 100 μg | 500 μg | 1 mg

100 ua

100 µg

100 µg

 Isotype:
 Mouse IgG2bλ

 Application:
 ELISA, ICC, FUNC (Blocking)

Functional Application

Mouse: Inhibits the binding of mouse angiopoietin-2 to mouse Tie-2. $ND_{50}^* = 50-60$ m/ml (for 10 m/ml of mouse angiopoietin-2) *Human:* Inhibits the binding of human angiopoietin-2 to human Tie-2. $ND_{50}^* = 8-12$ m/ml (for 10 m/ml of human angiopoietin-2)

*ND₅₀: = 50% neutralizing dose of antibody for a given concentration of ligand.

LIT: Elevated angiopoietin-2 level in patients with continuous-flow left ventricular assist devices leads to altered angiogenesis and is associated with higher nonsurgical bleed-ing: C.E. Tabit, et al.; Circulation 134, 141 (2016)

Also available:

anti-Angiopoietin-2 (human), mAb (rec.) (blocking) (Angy-1-4) Prod. No. AG-27B-0015



FIGURE: Antagonizing Angiopoietin-2 *in vivo* with anti-ANG-2, mAb (rec.) (blocking) (Angy-2-1) (AG-27B-0016PF) increases triglyceride levels.

METHOD: After High Fat Diet (HFD) challenges for five weeks in wild-type C57BL/6 mice, control IgG (left panel) or anti-ANG-2 (Clone Angy-2-1) blocking antibody (right panel) (4 µg/g body weight; twice/week) were administrated and afterwards the mice underwent metabolic analyses of the triglycerides levels from both groups.

🝳 anti-ADAM17 (human), mAb (rec.) (blocking) (D1(A12))

AG-27B-6000PF

Isotype Application F Preservative Free Human loG1 100 µg

Functional Application Inhibits human ADAM17 activity at 15µg/ml (200nM).

FUNC (Blocking)

LIT: Cross-domain inhibition of TACE ectodomain: C.J. Tape, et al.; PNAS 108, 5578 (2011) • Targeting ADAM-17 with an inhibitory monoclonal antibody has antitumour effects in triple-negative breast cancer cells: F. Caiazza, et al.; Br. J. Cancer 112, 1895 (2015)

Also available: anti-ADAM17 (human), mAb (rec.) (blocking) (D1(A12)) (Fab) (His)

Prod. No. AG-27B-6003PF



FIGURE: D1(A12) IgG inhibits constitutive shedding of TNF- α . from IGR0V1 (human ovarian cancer cell line) into culture medium. Medium was collected after 48 hours of incubation with or without IgGs at 200nM.

🞗 Works in Human 🛛 😰 Works in Mouse



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APPLICATIONS: FUNC: Functional Application; ICC: Immunocytochemistry; IHC: Immunohistochemistry; IP: Immunoprecipitation; WB: Western blot FORMULATION: PF = Preservative free



FuncAbs™

anti-BAFF (mouse), mAb (blocking) (Sandy-2)

Preservative Free

AG-20B-0063 AG-20B-0063PF

IsotypeMouse IgG1ApplicationIP, FUNC (Blocking)

Functional Application Inhibition of mouse BAFF binding to BAFF-R and TACI (BCMA not tested); blocks BAFF activity in mice.

LIT: Antibodies that block or activate mouse B cell activating factor of the TNF family (BAFF) respectively induce B cell depletion or B cell hyperplasia: C. Kowalczyk-Quintas, et al.; J. Biol. Chem. 291, 19826 (2016)

Also available:

anti-BAFF (human), mAb (blocking) (4.62)

Prod. No. AG-20B-0017



FIGURE: anti-BAFF (mouse), mAb (Sandy-2) (preservative free) (Prod. No. AG-20B-0063PF) blocks the action of endogenous BAFF *in vivo*.

METHOD: Wild type C57BL/6 mice were treated at day 0 (single administration) with monoclonal antibody anti-BAFF (mouse), mAb (Sandy-2) (preservative free) (at 2mg/kg). Lymph nodes were prepared at week 2 and analyzed by FACS for the presence of T (CD3) and B (CD19) cells. Untreated BAFF WT and KO mice were analyzed in parallel.

anti-BTLA (human), mAb (blocking) (6F4)

100 µg

Functional Application Inhibits interaction of BTLA to HVEM or UL144.

LIT: T Cell Intrinsic Heterodimeric Complexes between HVEM and BTLA Determine Receptivity to the Surrounding Microenvironment: T.C. Cheung, et al.; J. Immunol. 183, 7286 (2009)

100 μg 100 μg

🝳 anti-CD40L (human), mAb (rec.) (blocking) (hu5c8)

AG-27B-6002PF	Preservative Free

Application WB, FACS, FUNC (Blocking)

Application ELISA, FACS, FUNC (Blocking)

100 µg

100 µg

Functional Application Neutralizes CD40L function by blocking the interaction between CD40 and CD40L *in vitro* and *in vivo*.

LIT: Enhancement of T cell activation by immobilized hu5C8 (anti-CD40L) monoclonal antibody: M. Arpinati, et al.; Eur. J. Haematol. 80, 322 (2008)

anti-OX40L (human), mAb (rec.) (blocking) (R4930)

AG-27B-6001	PF Preservative Free
Application	FACS, FUNC (Blocking)

Functional Application Binds to the co-stimulatory human OX40L inhibiting its interaction with OX40 *in vitro* and *in vivo*.

LIT: OX40L blockade and allergen-induced airway responses in subjects with mild asthma: G.M. Gauvreau, et al.; Clin. Exp. Allergy 44, 29 (2014)

🔇 anti-LAG-3 (human), mAb (blocking) (17B4)

AG-20B-0012		100 µg
AG-20B-0012I	YF Preservative Free	100 µg
Application	FACS, ICC, IHC, IP, WB, FUNC (Blocking)	

Also available:

anti-LAG-3, mAb (blocking) (11E3) Prod. No. AG-20B-0011



Functional Application Blocks LAG-3/MHC class II interactions.

LIT: The negative regulatory function of the lymphocyte-activation gene-3 co-receptor (CD223) on human T cells: L. Macon-Lemaitre and F. Triebel; Immunology 115, 170 (2005)

🝳 anti-VEGF-A (human), mAb (3(6D3))

AG-20T-0105

Application ELISA, WB, FUNC (Blocking)

200 µg

Functional Application Inhibits VEGF-A signaling.

LIT: DLL1-mediated Notch activation regulates endothelial identity in mouse fetal arteries: I. Sörensen, et al.; Blood 113, 5680 (2009)



Blocking/Neutralizing Functional Antibodies

FuncAbs™

anti-IL-33 (mouse), mAb (rec.) (blocking) (Bondy-1-1)

AG-27B-0013 AG-27B-0013PF

Preservative Free

100 ua 100 µg | 500 µg | 1 mg

Isotype Mouse laG2b Application ELISA, FUNC (Blocking)

Functional Application

Inhibits the binding of mouse IL-33 to ST2/IL-1RAcP.

LIT: Regulation of de novo adipocyte differentiation through crosstalk between adipocytes and pre-adipocytes: T.D. Challa, et al.; Diabetes 64, 4075 (2015) • Male-specific IL-33 expression regulates sex-dimorphic EAE susceptibility: A.E. Russi, et al.; PNAS (epub ahead of print) (2018)

FIGURE: Binding of IL-33 (mouse) to ST2/IL-1RACP is inhibited by Bondy-1-1. IL-33 (mouse) was coated on an ELISA plate at 1µg/ml. Bondy-1-1 or an unrelated mAb (recombinant) (Control) were added (starting at 40µg/ml with a twofold serial dilution) together with 100µl of supernatant of cells containing ST2 (human):Fc/IL-1RACP (human):Fc. After incubation for 1 h at RT, the binding was detected using an anti-Fc human antibody (HRP).

anti-Netrin-1 (human), mAb (rec.) (blocking) (2F5)

AG-27B-0018PF

Application

lsotype

Preservative Free

Human IgG2λ

ELISA, FUNC (Blocking)

100 µg | 500 µg

Functional Application

Inhibits the activation of human and mouse Netrin-1 to human or mouse receptors DCC or UNC5 (KD antibody-Netrin-1 is 1.5nM).

LIT: Targeting netrin-1/DCC interaction in diffuse large B-cell and mantle cell lymphomas: T. Broutier, et al.; EMBO Mol. Med. 8, 96 (2016)

FIGURE: anti-Netrin-1 (human), mAb (rec.) (blocking) (2F5) (preservative free) (Prod. No. AG-27B-0018PF) blocks tumor growth in vivo. Method: Tumor cells (OCI-Ly3) were implanted in SCID mice by subcutaneous injection of 3x10⁶ cells in 100µl of PBS. When tumors reached 150mm³, mice received intraperitoneal injections of blocking anti-Netrin-1 mAb (2F5) at 20mg/kg or an equal volume of the antibody control anti-Netrin-1 (human), mAb (rec.) (H4) (preservative free) (Prod. No. AG-27B-0020PF) every two days. Tumor growth rates from the beginning of treatment are shown.

100 µg

anti-Zika Virus Envelope Protein (EIII domain), mAb (rec.) (neutralizing) (ZKA64)

AG-27B-6004PF **Preservative Free**

Application FUNC (Neutralizing)

LIT: Specificity, cross-reactivity, and function of antibodies elicited by Zika virus infection: K. Stettler, et al.: Science 353, 823 (2016)

Other Blocking Antibodies

See www.adipogen.com for More Information!

Neutralizes Zika Virus (ZIKV) with an IC₅₀ of ~93ng/

ml. Also enhances ZIKV infection in non-permissive

K562 cells at a broad range of concentrations (not

Functional Application

above $1\mu q/ml$).

ANTIBODIES	PID	APPLICATIONS	FUNCTIONAL APPLICATION
(IHC) anti-Fibronectin (EDA), mAb (blocking) (IST-9) (PF)	AG-20B-6001PF	ELISA, FUNC, ICC, IHC, WB	Inhibits binding of Fibronectin EDA domain to α 5- β 1, α 4- β 1 and α 9- β 1 integrins.
anti-Periostin, mAb (blocking) (OC-20) (PF)	AG-20B-6000PF	ICC, FUNC	Blocks interaction with the integrins $\alpha\nu\beta3$ and $\alpha\nu\beta5$. Inhibits angiogenesis and tumor growth and blocks allergen-induced inflammation <i>in vivo</i> in mice.
anti-NS5B (HCV), mAb (blocking) (5B-12B7)	AG-20B-0003	ICC, IP, FUNC	Blocks the RNA-dependent RNA polymerase activity in vitro.
anti-TRAIL-R1 (human), mAb (HS101) (PF)	AG-20B-0022PF	FACS, IP, ICC, FUNC	Inhibition/Neutralizing (blocks TRAIL-R1 mediated killing if applied in solution).
anti-TRAIL-R2 (human), mAb (HS201) (PF)	AG-20B-0023PF	FACS, IP, ICC, FUNC	Inhibition/Neutralizing (blocks TRAIL-R2 mediated killing if applied in solution).



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🞗 Works in Human 🛛 😰 Works in Mouse







anti-BAFF-R (mouse), mAb (9B9)

AG-20B-0034				
AG-20B-0034PF	Preservative Free			
AG-20B-0034B	Biotin			
Different labels available.				

IsotypeRat IgG2aApplicationELISA, IP, FUNC (Depletion)

Functional Application

Depletes B cells in vivo.

LIT: Crucial role for BAFF-BAFF-R signaling in the survival and maintenance of mature B cells: M. Rauch, et al.; PLoS ONE 4, e5456 (2009)



FIGURE: C57BL/6 mice were injected i.v. at day 0 with 0.5mg of 9B9. Absolute numbers of splenic T1 and T2/3 immature B cells, B-2 and MZ B cells, CD4 and CD8 T cells in controls (black bars) and 9B9 injected C57BL/6 mice at day 14 after injection (white bars). 5 mice were analyzed for each group.

anti-Neutrophils (mouse), mAb (blocking) (Nimp-R14)

AG-20B-0043 AG-20B-0043PF Preservative Free AG-20B-0043B Biotin Different labels available. 100 µg 500 µg | 2 mg | 10 mg 100 µg

100 µg

100 µg

100 µg

IsotypeRat IgG2aApplicationFACS, IHC, ICC, FUNC (Depletion)

Functional Application

Optimal reagent to deplete neutrophils in vivo (250 µg/mouse).

LIT: An immunomodulatory function for neutrophils during the induction of a CD4+ Th2 response in BALB/c mice infected with Leishmania major: F. Tacchini-Cottier, et al.; J. Immunol. **165**, 628 (2000)



FIGURE: Mouse neutrophils are depleted *in vivo* by Nimp-R14. Mice were injected i.p. with 250µg of Nimp-R14 (B) or with Control mAb (A) in BALB/c mouse 6 h prior to *Leishmania major* infection (3x106 parasites injected in the hind footpad). 3 days later, blood (100µl) was subjected to flow cytometric analysis after staining with APC/CY7-labeled anti-Ly66 antibody (clone 1A8).

The best depleting antibody for neutrophils in mice!



- Produced in mammalian cells (CHO or HEK 293).
- Similar properties compared to monoclonal antibodies developed in mice / rat (e.g. affinity in the low nanomolar range).

See Page 7 for More Information!

- Detect protein modifications (e.g. phosphorylations, ubiquitinations).
- Possibility to exchange the Fc region with Fc from other species.

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Inducing/Activating Antibodies

FuncAbs™

🙋 anti-LTβR (mouse), mAb (4H8 WH2)

AG-20B-0008 AG-20B-0008PF

Preservative Free

100 µg 100 µg

100 µg | 500 µg

100 µg | 500 µg

50 µg

100 µg

Isotype Rat lgG2a Application FACS, FUNC (Activation)

Functional Application for 4H8 WH2:

Agonists inducing BAFF, chemokines and integrins in vitro and in vivo.

LIT: LTBR Signaling Induces Cytokine Expression and Up-Regulates Lymphangiogenic Factors in Lymph Node Anlagen. M.F. Vondenhoff, et al.; J. Immunol. 182, 5439 (2009)

Also available: anti-LT^βR (mouse), mAb (3C8)

Prod. No. AG-20B-0041



FIGURE: Treatment of cultured WT MEFs with agonistic LTBR mAb (4H8 WH2), but not with an isotype matched control mAb, results in the up-regulation of IL-7 mRNA expression. MEFs were collected at 6, 24, and 30 h after stimulation with 4H8 WH2. Relative expression levels at t = 0 were set at 1,0. Experiments were performed three times. *, p < 0.05.

ANDARD

Control

FGK45

anti-CD40 (mouse), mAb (FGK45)

AG-20B-0036 AG-20B-0036PF

Preservative Free

Isotype Rat lgG2a FACS, FUNC (Activation) Application

Functional Application

Activates B and NK cells in vitro and in vivo.

LIT: Ovarian insufficiency and early pregnancy loss induced by activation of the innate immune system: A. Erlebacher, et al.; J. Clin. Invest. 114, 39 (2004)

FIGURE: Systemic immune activation by CD40 ligation. Mice were sacrificed on day 8 after daily treatment on day 4-7 with FGK45 or control. FGK45 treatment, elevated splenocyte numbers in both groups. *P < 0.005. Data represent mean \pm SD for three to four mice per group.

anti-Fas (human), mAb (APO-1-3)

AG-20B-0062PF

Preservative Free

Mouse IgG3 lsotype FACS, IP, WB, FUNC (Activation) Application

Functional Application

Induces apoptosis with or without cross-linking (Protein A), depending on cell type.

LIT: Monoclonal antibody-mediated tumor regression by induction of apoptosis: B.C. Trauth, et al.; Science 245, 301 (1989)



450

400

300

250

200 Splenocyte

150

numbers (x 10⁻⁶) 350

FIGURE: Induction of growth Inhibition by apoptosis by APO-1-3 or control medium. SKW6.4 cells were pre-incubated with APO-1-3 (100 nglml). ['H)TdR incorporation was measured.



🞗 Works in Human 🛛 😰 Works in Mouse

anti-Lewis^{y/b} hapten (human), mAb (SC104)

AG-20B-6002PF

Preservative Free

Application ELISA, FACS, ICC, IHC, FUNC (Inducing)

LIT: Development of second generation monoclonal antibodies recognising Lewisy/b antigen by anti-idiotypic immunisation: L.G. Durrant, et al.; Hybridoma 12, 647 (1993) • A new anticancer glycolipid monoclonal antibody, SC104, which directly induces tumor cell apoptosis: L.G. Durrant, et al.; Cancer Res. 66, 5901 (2006)



Directly induces tumor-specific cell death without the need for immune effector cells by induction of caspase-mediated apoptosis. Optimal concentrations used to induce tumor cell death in vitro and in vivo are between 10-30µg/ml.



6

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APPLICATIONS: FUNC: Functional Application; ICC: Immunocytochemistry; IHC: Immunohistochemistry; IP: Immunoprecipitation; WB: Western blot FORMULATION: PF = Preservative free



ANDARD



Treatment time (days)

Recombinant Monoclonal Antibodies [RecMAbs™]

Antibody phage display is an in vitro technology to generate recombinant monoclonal antibodies (RecMAbs[™]). It is an alternative to the hybridoma technology, since it circumvents the limitations of the immune system. Antibodies developed by "antibody phage display technology" use human naïve antibody gene libraries. These libraries consist of billions of scFv (single chain fragment variable) composed of VH (variable domain of the human immunoglobulin heavy chain) and VL (variable domain of the human immunoglobulin light chain) connected by a polypeptide linker. The antibody fragments are fused to the coat protein pIII and displayed on the surface of filamentous bacteriophages (M13). The scFvs are selected *in vitro* by affinity selection on the antigen in a process termed panning, where the antigen of interest is coated on a vial (see Figure). Panning methods are based on four major steps: i) preparation of phage-displaying libraries; ii) adsorbing the specific binding phage, iii) removal of non-specific or low affinity phages, and recovering of target binders, that will be reamplified after bacteria infection for the next round of selection. Multiple rounds of panning are performed to enrich for the antigenspecific scFv-phages. Monoclonal antibodies are subsequently identified by screening after the last round of selection. The selected monoclonal scFv is cloned into an appropriate vector containing a Fc portion of interest and then produced in mammalian cells to generate an IgG like scFv-Fc fusion protein.

There are many advantages to use recombinant antibodies instead of classical antibodies: i) economical production and permanent storage of DNA clones are some of the assets of the recombinant antibody approach; ii) absence of requirement of sacrificing animals in large animal facility; iii) use of a single stable antibody fragment makes it straightforward to reformat a RecMAb[™] into a full-length IgG construct or a single chain fragment variable (Fv).

An important attribute of the RecMAbs[™] phage display approach is the ability to design selection strategies to generate antibodies with customized functions (FuncAbs[™]), which furthermore can be classified based on activity (see frontpage) or mode of binding. For instance, it is possible to generate RecMAbs[™] that: (1) preferentially recognize a specific conformational state and thus, have the potential to induce a specified conformational change; (2) target specific regions of the surface of the target protein ("regio-specific") or (3) specifically recognize multi-protein complexes. Production of Recombinant Monoclonal Antibodies (RecMAbs™)





AdipoGen[®]

Conformation-specific Recombinant Antibodies

anti-Rab1-GTP, mAb (rec.) (ROF7)

AG-27B-0006

lsotype Human IgG2bλ Application ICC, IP Specificity Hu, Ms, Rt, Dg

LIT: Characterization of single chain antibody targets through yeast two hybrid: O. Vielemeyer, et al.; BMC Biotechnol. 10, 59 (2010)

FIGURE: Rab1-GTP is detected by immunocytochemistry using ROF7. Picture courtesy of Dr. Sandrine Moutel & Dr. Franck Perez Lab, Curie Institute, Paris.

anti-Rab6-GTP, mAb (rec.) (AA2)

AG-27B-0004 AG-27B-0004TD

ATTO 488

100 µg 100 µg

100 µg

Human IgG2bλ Isotype Application ICC, WB Specificity Hu, Ms, Dr

LIT: Recombinant antibodies to the small GTPase Rab6 as conformation sensors: C. Nizak, et al.; Science 300, 984 (2003)

FIGURE: Rab6-GTP is detected by immunocytochemistry using AA2. Picture courtesy of Dr. Sandrine Moutel & Dr. Franck Perez Lab, Curie Institute, Paris.

anti-Tubulin-GTP, mAb (rec.) (MB11)

AG-27B-0009

lsotype	Human IgG2bλ
Application	ICC, WB
Specificity	Hu, Ms, Rt, Dr

LIT: Detection of GTP-Tubulin Conformation in Vivo Reveals a Role for GTP Remnants in Microtubule Rescues: A. Dimitrov, et al.; Science 322, 1353 (2008)

FIGURE: Tubulin-GTP is detected by immunocytochemistry using MB11. Picture courtesy of Dr. Sandrine Moutel & Dr. Franck Perez Lab, Curie Institute, Paris

Other Recombinant Monoclonal Antibodies

ANTIBODIES	PID	SIZE	ISOTYPE	APPLICATIONS	SPECIES
NEW anti-LRP5/6, mAb (rec.) (Heldy-1-4)	AG-27B-0019	100 µg	Human IgG2λ	FACS	Hu, Ms
anti-Giantin, mAb (rec.) (TA10)	AG-27B-0003	100 µg	Human IgG2λ	ICC	Hu, Ms
anti-Giantin, mAb (rec.) (TA10) (ATTO 488)	AG-27B-0003TD	100 µg	Human lgG2 λ	ICC	Hu, Ms
anti-Myosin IIA (non-muscle) (heavy chain), mAb (rec.) (SF9)	AG-27B-0010	100 µg	Human lgG2λ	EM, ELISA, ICC, WB	Hu, Ms, Rt, Dr
anti-Myosin IIA (non-muscle) (heavy chain), mAb (rec.) (SF9) (ATTO 488)	AG-27B-0010TD	100 µg	Human lgG2λ	ICC	Hu, Ms, Rt, Dr
anti-HMGB1, mAb (rec.) (Giby-1-4)	AG-27B-0002	100 µg	Human IgG2λ	ELISA, WB	Hu, Ms, Rt
anti-HMGB1, mAb (rec.) (Giby-1-4) (Biotin)	AG-27B-0002B	100 µg	Human lgG2λ	ELISA, WB	Hu, Ms, Rt
anti-IL-1R2 (mouse), mAb (rec.) (Praxy-1-1)	AG-27B-0011	100 µg	Human lgG2 λ	ELISA, FACS	Ms
anti-IL-33 (mouse), mAb (rec.) (Carly-1-4)	AG-27B-0012	100 µg	Human lgG2 λ	ELISA, WB	Ms
anti-PEDF (human), mAb (rec.) (Serpy-1-4)	AG-27B-0014	100 µg	Human lgG2 λ	ELISA, WB	Hu
anti-EGFP, mAb (rec.) (G3)	AG-27B-0007	100 µg	Human IgG2λ	ELISA, ICC, IP	N/A

SPECIES: Hu = Human; Ms = Mouse; Rt = Rat; Dg = Dog; Dr = Drosophila

🝳 Works in Human 🛛 😰 Works in Mouse









RecMAbs[™]

100 µg

The Tubulin Code: Post-translational Modifications of Tubulins

Post-translational modifications (PTMs) are highly dynamic and often reversible processes where protein functional properties are altered by addition of a chemical group or another protein to its amino acid residues. As key cytoskeletal proteins with roles in neuronal development, growth, motility and intracellular trafficking, tubulins and microtubules (MTs) are major substrates for PTMs. They include tyrosination/detyrosination, $\Delta 2$ -tubulin formation, **acetylation**, phosphorylation, polyamination, ubiquitination, **polyglutamylation** and glycylation (**see Figure**). Most of these PTMs preferentially take place on tubulin subunits already incorporated into microtubules.

PTMs are involved in fine-tuning of interactions between microtubules and different MT-interacting proteins. Most axonal microtubules are detyrosinated and further labeled with acetate and polyglutamate marks. By contrast, the unstable microtubules are enriched in carboxy-terminal tyrosination and devoid of glutamate tails. Detyrosination and polyglutamylation of MTs can selectively modulate the affinities and motility of molecular motors. Acetylation seems to control intracellular transport by regulating the traffic of kinesin motors. Microtubules PTMs deregulation have impact on neuronal development and diseases.



FIGURE: Tubulin PTM Overview. Adapted from C. Janke; J. Cell. Biol. 206, 461 (2014)

Recombinant Microtubule-target Antibodies

RecMAbs[™]

IINIQUE

ANTIBODIES	PID	SIZE	ISOTYPE/SOURCE	APPLICATION	SPECIES
anti-Tubulin-GTP, mAb (rec.) (MB11) (UNIQUE)	AG-27B-0009	100 µg	Human lgG2λ	ICC	Hu, Ms, Rt, Dr
anti- $lpha$ -Tubulin, mAb (rec.) (F2C)	AG-27B-0005	100 µg	Human lgG2λ	ICC, WB	Hu, Ms, Bv
anti- α -Tubulin, mAb (rec.) (F2C) (ATTO 488)	AG-27B-0005TD	100 µg	Human lgG2λ	ICC	Hu, Ms, Bv
anti-β-Tubulin, mAb (rec.) (S11B)	AG-27B-0008	100 µg	Rabbit	ELISA, ICC, WB	Hu, Ms, Rt, Pg, Dr, Mk

Validated Post-translational Modification-specific Antibodies

ANTIBODIES	PID	SIZE	ISOTYPE/SOURCE	APPLICATION
anti- $lpha$ -Tubulin (acetylated), mAb (TEU318)	AG-20B-0068	100 µg	Mouse IgG1	ICC, WB
anti-Polyglutamylation Modification, mAb (GT335)	AG-20B-0020	100 µg	Mouse IgG1ĸ	EM, IHC, ICC, IP, WB
anti-Polyglutamylation Modification, mAb (GT335) (Biotin)	AG-20B-0020B	100 µg	Mouse IgG1ĸ	ICC, IHC, IP, WB
anti-Polyglutamate chain (polyE), pAb (IN105)	AG-25B-0030	50 µg	Rabbit	ICC, IHC, WB
NEW anti-Tubulin (glycylated), pAb (Gly-pep1)	AG-25B-0034	100 µg	Rabbit	ICC, IP, WB
NEW anti-PSD-95 (palmitoylated), mAb (rec.) (PF11)	AG-27B-0021	100 µg	Human IgG2	ICC, IHC



FIGURE: Immunofluorescence staining using anti-Tubulin (glycylated), pAb (Gly-pep1) (Prod. No. AG-25B-0034) of multiciliated ependymal cells and cells with primary cilia. Method: Radial glial cells isolated from newborn wildtype mice, and the MDCK cell line were serum starved to induce ciliogenesis. Cells were fixed with a microtubule-stabilizing protocol and stained with anti-Tubulin (glycylated), pAb (Gly-pep1) (1:5,000; red), anti-α-Tubulin (acetylated), mAb (TEU318) (AG-20B-0068) (green) and DAPI (blue). Gly-pep1 staining is observed specifically on the cilia. *Picture courtesy of Sudarshan Gadadhar* and Carsten Janke, Institut Curie



FIGURE: Palmitoylated PSD95 is detected by immunocytochemistry using anti-PSD-95 (palmitoylated), mAb (rec.) (PF11) (Prod. No AG-27B-0021). Method: HeLa cells were cotransfected with DHHC2 (palmitoylating enzyme) + PSD95-GFP (A) or DHHC2 (palmitoylating enzyme) alone (B). Cells in "B" were fixed with paraformaldehyde (3%), permeablized in PBS-H SA 0.2 % + Saponin 0.05 % and incubated with anti-PSD-95 (palmitoylated), mAb (rec.) (PF11) (1µg/ml in PBS-BSA-Saponin). After incubation for 30min at RT and several washes in PBS, cells are treated with a goat anti-human (ATT0488) antibody in PBS-BSA-Saponin for 30min at RT, washed and mounted in Moewiol. Nuclei are stained with DAPI. Merge image is shown at the right. *Picture courtesy of Dr. Moutel*





Functional Antibodies

Blocking/Neutralizing Antibodies [FuncAbs[™]]

PRODUCT NAME	PID (*)	APPLICATIONS	FUNCTIONAL APPLICATION
CD4 (human), mAb (QS4120)	ANC-147	FUNC, FACS, ELISA	Blocks binding of HIV-1 gp120 protein to CD4 and also blocks HLA Class II rosette formation.
CD11a (human), mAb (38)	ANC-158	FUNC, FACS, WB	Blocks binding of ICAM-1 and ICAM-3 to LFA-1 at 5-10 $\mu\text{g/ml}.$
CD11b (human), mAb (ICRF44)	ANC-159	FUNC, FACS	Blocks homotypic neutrophil and monocyte (FMLP induced) aggregation.
CD16 (human), mAb (3G8)	ANC-165	FUNC, FACS	Blocks binding of complexed IgG to CD16.
CD18 (human), mAb (IB4)	ANC-167	FUNC, FACS	Blocks binding of ICAM-1 and ICAM-3 to LFA-1.
CD21 (human), mAb (BU33)	ANC-170	FUNC, FACS, WB	Inhibits binding to CD23.
CD31 (human), mAb (158-2B3)	ANC-180	FUNC, FACS	Blocks homophilic interaction and heterophilic transendothelial migration.
CD32 (human), mAb (7.3)	ANC-181	FUNC, FACS	Blocks immune complex binding.
CD40L [CD154] (human), mAb (24-31)	ANC-353	FUNC, FACS, ELISA, IHC, WB	Blocks MLR, sgp39 induced human B cell proliferation and T cell dependent B cell differentiation.
CD44 (human), mAb (BU75)	ANC-352	FUNC, FACS, WB	Blocks binding of HA to CD44.
CD49d (human), mAb (BU49)	ANC-200	FUNC, FACS	Blocks VLA-4 binding to VCAM-1. It can be used to aid in purification of FoxP3 ⁺ Treg cells. Induces IL-8 production by U-937 cells.
CD50 (human), mAb (186-2G9)	ANC-201	FUNC, FACS	Blocks binding of CD11a (LFA-1) to CD50 (ICAM-3).
CD54 (D1) (human), mAb (15.2)	ANC-205	FUNC, FACS, ELISA, WB	Inhibits CD54 binding to LFA-1.
CD54 (D2) (human), mAb (8.4A6)	ANC-206	FUNC, FACS, ELISA	Inhibits CD54 binding to LFA-1.
CD58 (human), mAb (TS2)	ANC-210	FUNC, FACS	Inhibits HLA-DR mediated T cell cytotoxicity.
CD64 (human), mAb (10.1)	ANC-216	FUNC, FACS, WB	Blocks binding of $Fc\gamma RI$ to immunoglobulin opsonized cells.
CD70 (human), mAb (BU69)	ANC-222	FUNC, FACS, ELISA, ICC, IHC	Inhibits T cell proliferation induced by dendritic cells.
CD62E (human), mAb (HAE-1f)	ANC-240	FUNC, FACS	Blocks the function of CD62E.
CD62L (human), mAb (LAM 1-116)	ANC-261	FUNC, FACS	Blocks CD62L function and induces expression of β -1 and β -2 integrins.
CD80 (human), mAb (P1.H1.A1.A1)	ANC-110	FUNC, FACS, ELISA	Blocks binding of soluble CD152 lg fusion protein to CD80.
CD86 (human), mAb (BU63)	ANC-307	FUNC, FACS	Blocks MLR and blocks binding of soluble CD152-mouse Ig fusion protein to CD86.
CD94 (human), mAb (HP-3D9)	ANC-315	FUNC, FACS	Inhibits IL-2 dependent proliferation of NK cells.

(*) The Ancell Product # is build by the prefix (ANC-), main PID (3 digits) and a suffix (3 digits). The last 3 digits define the labels: -020 = Preservatives | -820 = Preservative Free | -030 = Biotin | -040 = FITC | -050 = R-PE | -060 = APC | -520 = F(ab')2 | -580 = Fab | -070 = PE-Cy7 | -350 = DyLight350 FAB: Fragment Antigen Binding; FACS: Flow Cytometry; FUNC: Functional Application; ICC: Immunocytochemistry; IHC: Immunohistochemistry; IP: Immunoprecipitation; WB: Western Blot





Blocking/Neutralizing Antibodies [FuncAbs[™]]

continued

PRODUCT NAME	PID (*)	APPLICATIONS	FUNCTIONAL APPLICATION
CD104 (human), mAb (UMA 9)	ANC-325	FUNC, FACS, WB	Partially blocks binding to laminin.
CD106 (human), mAb (1.G11B1)	ANC-327	FUNC, FACS, ELISA, IHC, WB	Blocks leukocyte adhesion.
CD122 (human), mAb (9A2)	ANC-343	FUNC, FACS	Inhibits binding of IL-2 to IL-2R β (CD122).
CD137 (human), mAb (4B4-1)	ANC-360	FUNC, FACS, ELISA	Blocks binding of CD137-human Ig fusion protein to Raji cells.
CD147 (human), mAb (UM-8D6)	ANC-376	FUNC, FACS, IP, WB	Inhibits homotypic aggregation, adhesion to matrix proteins and migration through matrigel.
CD152 (human), mAb (ANC152.2/8H5)	ANC-359	FUNC, FACS, ELISA	Blocks binding of CD152 (CTLA-4)- human lg fusion protein to its CD80/CD86 receptor.
CD165 (human), mAb (AD2)	ANC-392	FUNC, FACS	Blocks the function of CD165.
CD166 (human), mAb (3A6)	ANC-393	FUNC, FACS	Blocks binding of CD6 to CD166.
CD252 (human), mAb (ANC10G1)	ANC-400	FUNC, FACS, ELISA	Blocks binding of recombinant CD134-mouse lg fusion protein.
CD257 (human), mAb (ANC2H3)	ANC-266	FUNC, ELISA	Blocks binding of recombinant human CD257(BAFF) to receptors on Raji cells in flow cytometry.
CD272 (human), mAb (ANC6E9)	ANC-272	FUNC, FACS, ELISA	Blocks binding of biotinylated CD270(HVEM)-mouse Ig fusion protein to CD272-mouse Ig fusion protein in EIA.
CD278 (human), mAb (ANC6C6)	ANC-265	FUNC, FACS, ELISA	Blocks binding of recombinant GL50-mouse Ig fusion protein to HPB-MLT cells.
TNF- α (human), mAb (J1D9)	ANC-398	FUNC, FACS, WB	Neutralizes TNF- α biological activities.

Activating/Inducing Antibodies [FuncAbs[™]]

PRODUCT NAME	PID (*)	APPLICATIONS	FUNCTIONAL APPLICATION
CD3 (human), mAb (UCHT1)	ANC-144	FUNC, FACS, WB	Activates T cells expressing CD3ε.
CD6 (human), mAb (3F7B6)	ANC-151	FUNC, FACS, WB	Activates T cells.
CD7 (human), mAb (3A1E)	ANC-152	FUNC, FACS	Induces T cell transmembrane calcium flux.
CD15 (human), mAb (AHN1.1)	ANC-164	FUNC, FACS, IHC	Activates normal monocytes and inhibits neutrophil chemotaxis.
CD19 (human), mAb (BU12)	ANC-168	FUNC, FACS	Induces adhesion of B cells.
CD28 (human), mAb (ANC28.1/5D10)	ANC-177	FUNC, FACS, ELISA	Stimulates expression of IL-2 from CD28 ⁺ cells.
CD40 (human), mAb (BE-1)	ANC-189	FUNC, FACS, ELISA, IP	Partially activates B cells.
CD40 (human), mAb (EA-5)	ANC-300	FUNC, FACS, ELISA	Partially activates B cells.
CD43 (human), mAb (DFT1)	ANC-192	FUNC, FACS, WB	Partially induces apoptosis in hemopoietic progenitor cells and also induces homopoietic aggregation.
CD49d (human), mAb (BU49)	ANC-200	FUNC, FACS	Blocks VLA-4 binding to VCAM-1. It can be used to aid in purification of FoxP3 ⁺ Treg cells. Induces IL-8 production by U-937 cells.
CD60 (human), mAb (UM4D4)	ANC-212	FUNC, FACS, WB	Activates T cells.
CD79b (human), mAb (SN8)	ANC-301	FUNC, FACS, WB	Induces signal transduction in B cells.
CD105 (human), mAb (SN6)	ANC-326	FUNC, FACS, IHC	Augments binding of TGF- β 1 to CD105 expressing leukemia cells.
lgM (human), mAb (UCHB1)	ANC-141	FUNC, FACS, ELISA	Delivers a costimulatory signal to B cells in vitro.

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anti-PD-1 (mouse), mAb (blocking) (1H10)

AG-20B-0075 AG-20B-0075PF

Preservative Free

Isotype Rat lgG2ak FACS, FUNC (Blocking) Application

anti-Asc, pAb (AL177)

Rabbit

Preservative Free

ATTO 647N

Functional Application

Blocks PD-1 binding. Induces a rapid activation and proliferation of T cells at concentration of 0.25µg/2x105 cells.

LIT: Antibody-mediated signalling through PD-1 costimulates T cells and enhances CD28dependent proliferation: M.L. del Rio, et al.; Eur. J. Immunol. 35, 3545 (2005)

Validated Inflammasome Antibodies

30 25 20 15 10 5 0 Control la 1H10 1H10 +anti-CD28

FIGURE: PD-1 receptor-induced CD4T cell activation and proliferation by PD-1 (mouse), mAb (blocking) (1H10) (AG-20B-0075). METHOD: Magnetic bead affinity purified CD4+ T cells from C57BL/6 mice are stimulated in vitro with PD-1 (mouse), mAb (blocking) (1H10), anti-CD28 and rat IqG2a isotype (control Iq) (0.25µq/ 2x10⁵ cells) for 48h. Proliferation is determined by [3H]thymidine incorporation. The presence of anti-CD28 mAb increases 1H10 mAb-mediated proliferation.

100 µg

100 µg | 500 µg

(Prod. No. AG-25B-0006). Total protein extracts from various human (293-T, Jurkat, Raj, Ramos, BJAB, THP-1, U937, K562, Raw, HeLa) and mouse (EL-4, A20) cell lines were run on SDS-PAGE and Pycard detected by anti-Asc, pAb (AL177) at 1:1'000 dilution. Anti-rabbit

MW (kDa) 25 -

FIGURE: Western blot analysis of human and mouse cell lines using anti-Asc, pAb (AL177) IgG coupled horse radish peroxidase was used at 1:5'000 dilution for ECL detection.

NDARDS



anti-Caspase-1 (p20) (mouse), mAb (Casper-1)

AG-20B-0042 AG-20B-0042B **Biotin**

AG-25B-0006

Application

Isotype

AG-25B-0006PF

AG-25B-0006TS

100 µg 100 µg

100 µg

wildtype NLRP3-/- CASP1-/-OF LO

Pycard

Splice-varian

of Pycard

Isotype Mouse IgG1 WB (1µg/ml) (see online protocol), IHC (PS), IP Application

ICC, IHC, IP, WB, FUNC (Inhibition)

FIGURE: Mouse caspase-1 (p20) is detected by immunoblotting using anti-Caspase-1 (p20) (mouse), mAb (Casper-1) (Prod. No. AG-20B-0042).

METHOD: Caspase-1 was analyzed by Western blot in cell extracts and supernatants of differentiated bone marrow-derived dendritic cells (BMDCs) from wild-type, NLRP3-/- and caspase-1-/- mice activated or not by 5µM nigericin (Prod. No. AG-CN2-0020) for 30 min. Cell extracts and supernatants were separated by SDS-PAGE under reducing conditions, transferred to nitrocellulose and incubated with anti-Caspase-1 (p20) (mouse), mAb (Casper-1) (1µg/ml). Proteins were visualized by a chemiluminescence detection system.

anti-NLRP3/NALP3, mAb (Cryo-2)

AG-20B-0014

Mouse IgG2b Isotype Application ICC, IHC, IP, WB (1µg/ml) (see online protocol)

FIGURE: Mouse NLRP3 is detected in mouse macrophages using the monoclonal antibody to NLRP3 (Cryo-2) (Prod. No. AG-20B-0014).

METHOD: Cell extracts from mouse macrophages (BMDMs) WT (+/+) (lane 1), NLRP3 +/- (lane 2) or NLRP3 -/- (lane 3) with or without treatment with LPS (Song/ml) for 3h, were separated by SDS-PAGE under reducing conditions, transferred to nitrocellulose and incubated with the mAb to NLRP3 (Cryo-2) (1µg/ml). Proteins are visualized by a chemiluminescence detection system.



2 Works in Mouse



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