AdipoGen® LIFE SCIENCES

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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.

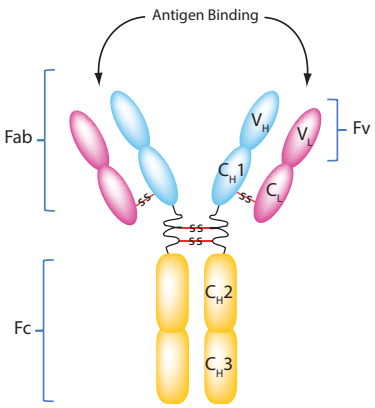
Antibodies are produced by: a) Injecting an antigen into mammals (mouse, rat, rabbit, goat, etc). Blood isolated from these animals contains **polyclonal antibodies** (multiple antibodies that bind to different epitopes of the same antigen), which are purified. b) Hybridoma technology generating **monoclonal 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).

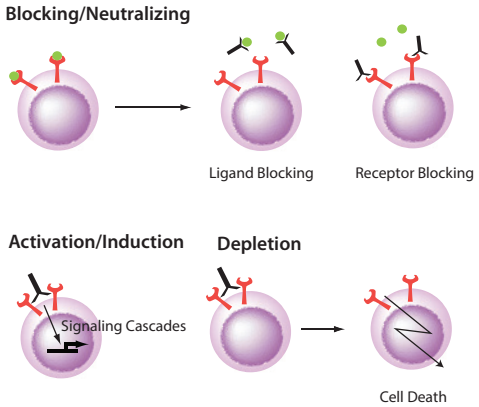
Functional Grade Antibodies (FuncAbs™):

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*.**

General Antibody Schematic



Different Types of Antibody Functionality



CONTENTS

Blocking/Neutralizing Functional Antibodies

UNIQUE

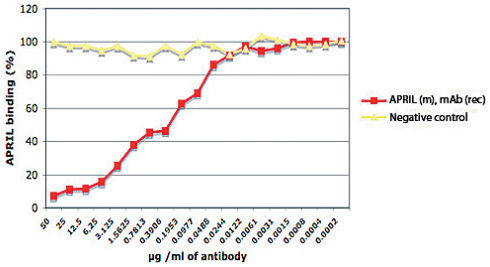
anti-APRIL (mouse), mAb (rec.) (blocking) (Apry-1-1)

AG-27B-0001		100 µg
AG-27B-0001PF	Preservative Free	100 µg
AG-27B-0001B	Biotin	100 µg

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)



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

Prod. No. AG-27B-0017

POTENT

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

AG-27B-0016		100 µg
AG-27B-0016PF	Preservative Free	100 µg | 500 µg | 1 mg

Isotype: Mouse IgG2bλ
Application: ELISA, ICC, FUNC (Blocking)

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

*ND_{50}: = 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 bleeding: C.E. Tabit, et al.; Circulation **134,** 141 (2016)

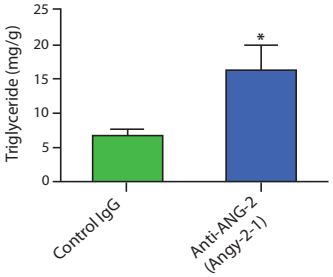


FIGURE: Antagonizing Angiopoietin-2 *in vivo* with anti-ANG-2, mAb (rec.) (blocking) (Angy-2-1) (AG-27B-0016PF) increases triglyceride levels.

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.

Also available:
anti-Angiopoietin-2 (human), mAb (rec.) (blocking) (Angy-1-4)

Prod. No. AG-27B-0015

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

AG-27B-6000PF	Preservative Free	100 µg

Isotype Human IgG1
Application FUNC (Blocking)

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

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)

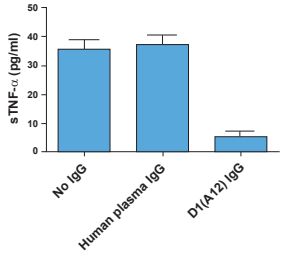


FIGURE: D1(A12) IgG inhibits constitutive shedding of TNF-α from IGROV1 (human ovarian cancer cell line) into culture medium. Medium was collected after 48 hours of incubation with or without IgGs at 200nM.

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

Prod. No. AG-27B-6003PF

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Works in Human Works in Mouse

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

UNIQUE

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

AG-20B-0063 100 µg
AG-20B-0063PF Preservative Free 100 µg

Isotype Mouse IgG1
Application IP, 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)

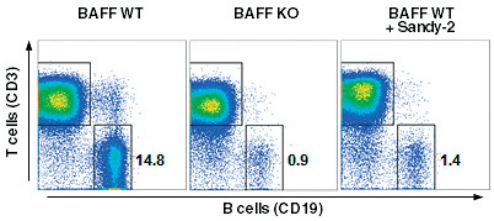

FIGURE: anti-BAFF (mouse), mAb (Sandy-2) (preservative free) (Prod. No. AG-20B-0063PF) blocks the action of endogenous BAFF *in vivo.*

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.

Also available:

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

Prod. No. AG-20B-0017

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

AG-20B-0049 100 µg

Application ELISA, FACS, FUNC (Blocking)

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)

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

AG-27B-6002PF Preservative Free 100 µg

Application WB, FACS, FUNC (Blocking)

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-6001PF Preservative Free 100 µg

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)

IHC GRADE

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

AG-20B-0012 100 µg
AG-20B-0012PF Preservative Free 100 µg

Application FACS, ICC, IHC, IP, WB, FUNC (Blocking)

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

Also available:

anti-LAG-3, mAb (blocking) (11E3)

Prod. No. AG-20B-0011

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 200 µg

Application ELISA, WB, FUNC (Blocking)

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

UNIQUE

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

AG-27B-0013		100 µg
AG-27B-0013PF	Preservative Free	100 µg | 500 µg | 1 mg

Isotype Mouse IgG2b
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)

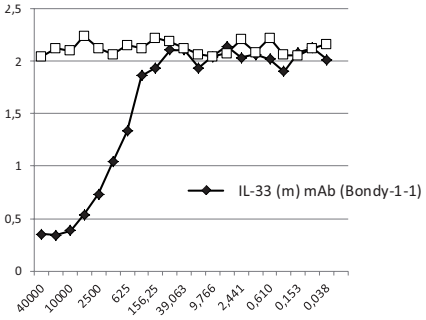

FIGURE: Binding of IL-33 (mouse) to ST2/IL-1RAcP is inhibited by Bondy-1-1. IL-33 (mouse) was coated on an ELISA plate at 1µg/ml. Bondy-1-1 or an unrelated mAb (recombinant) (Control) were added (starting at 40µg/ml with a twofold serial dilution) together with 100µl of supernatant of cells containing 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	Preservative Free	100 µg | 500 µg

Isotype Human IgG2λ
Application ELISA, FUNC (Blocking)

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)

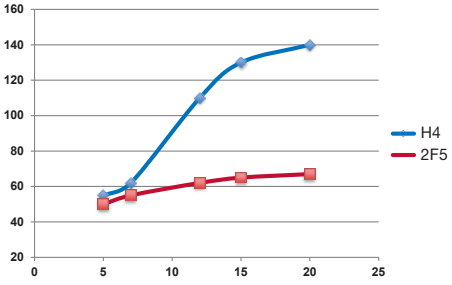

FIGURE: anti-Netrin-1 (human), mAb (rec.) (blocking) (2F5) (preservative free) (Prod. No. AG-27B-0018PF) blocks tumor growth *in vivo*.Method: Tumor cells (OCI-Ly3) were implanted in SCID mice by subcutaneous injection of $3x10^6$ cells in 100µl of PBS. When tumors reached $150mm^3$, mice received intraperitoneal 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.

NEW

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

AG-27B-6004PF	Preservative Free	100 µg

Application FUNC (Neutralizing)

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

Functional Application
Neutralizes Zika Virus (ZIKV) with an IC_{50} of ~93ng/ml. Also enhances ZIKV infection in non-permissive K562 cells at a broad range of concentrations (not above 1µg/ml).

Other Blocking Antibodies

See www.adipogen.com for More Information!

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 α5-β1, α4-β1 and α9-β1 integrins.
anti-Periostin, mAb (blocking) (OC-20) (PF)	AG-20B-6000PF	ICC, FUNC	Blocks interaction with the integrins αvβ3 and αvβ5. Inhibits angiogenesis and tumor growth and blocks allergen-induced inflammation *in vivo* 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

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

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

AG-20B-0034		100 µg
AG-20B-0034PF	Preservative Free	100 µg
AG-20B-0034B	Biotin	100 µg

Different labels available.

Isotype Rat IgG2a
Application ELISA, 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)

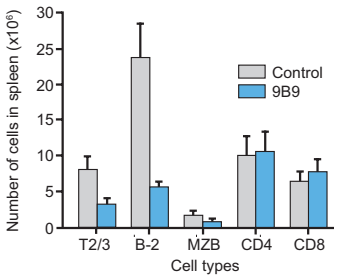

FIGURE: C57BL/6 mice were injected i.v. at day 0 with 0.5mg of 9B9. Absolute numbers of splenic T1 and T2/3 immature B cells, B-2 and MZ B cells, CD4 and CD8 T cells in controls (black bars) and 9B9 injected C57BL/6 mice at day 14 after injection (white bars). 5 mice were analyzed for each group.

POTENT

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

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

Different labels available.

Isotype Rat IgG2a
Application FACS, 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)

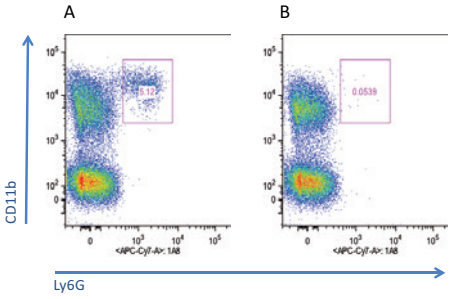

FIGURE: Mouse neutrophils are depleted *in vivo* by Nimp-R14. Mice were injected i.p. with 250µg of Nimp-R14 (B) or with Control mAb (A) in BALB/c mouse 6 h prior to *Leishmania major* infection (3x106 parasites injected in the hind footpad). 3 days later, blood (100µl) was subjected to flow cytometric analysis after staining with APC/CY7-labeled anti-Ly6G antibody (clone 1A8).

The best depleting antibody for neutrophils in mice!

Inducing/Activating Antibodies

anti-LTβR (mouse), mAb (4H8 WH2)

AG-20B-0008		100 µg
AG-20B-0008PF	Preservative Free	100 µg

Isotype Rat IgG2a
Application FACS, FUNC (Activation)

Functional Application for 4H8 WH2:
Agonists inducing BAFF, chemokines and integrins *in vitro* and *in vivo*.

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

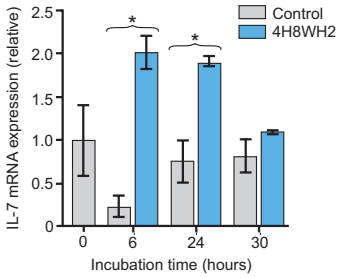

FIGURE: Treatment of cultured WT MEFs with agonistic LTβR mAb (4H8 WH2), but not with an isotype matched control mAb, results in the up-regulation of IL-7 mRNA expression. MEFs were collected at 6, 24, and 30 h after stimulation with 4H8 WH2. Relative expression levels at t = 0 were set at 1,0. Experiments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

Also available:

anti-LTβR (mouse), mAb (3C8)

Prod. No. AG-20B-0041

THE STANDARD

anti-CD40 (mouse), mAb (FGK45)

AG-20B-0036		100 µg | 500 µg
AG-20B-0036PF	Preservative Free	100 µg | 500 µg

Isotype Rat IgG2a
Application FACS, FUNC (Activation)

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)

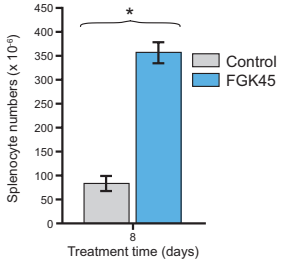

FIGURE: Systemic immune activation by CD40 ligation. Mice were sacrificed on day 8 after daily treatment on day 4-7 with FGK45 or control. FGK45 treatment, elevated splenocyte numbers in both groups. *$P < 0.005$. Data represent mean ± SD for three to four mice per group.

THE STANDARD

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

AG-20B-0062PF	Preservative Free	50 µg

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

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)

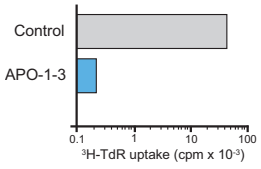

FIGURE: Induction of growth Inhibition by apoptosis by APO-1-3 or control medium. SKW6.4 cells were pre-incubated with APO-1-3 (100 ngIml). ['H)TdR incorporation was measured.

NEW

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

AG-20B-6002PF	Preservative Free	100 µg

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)

Functional Application
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.

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Works in Human Works in Mouse

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

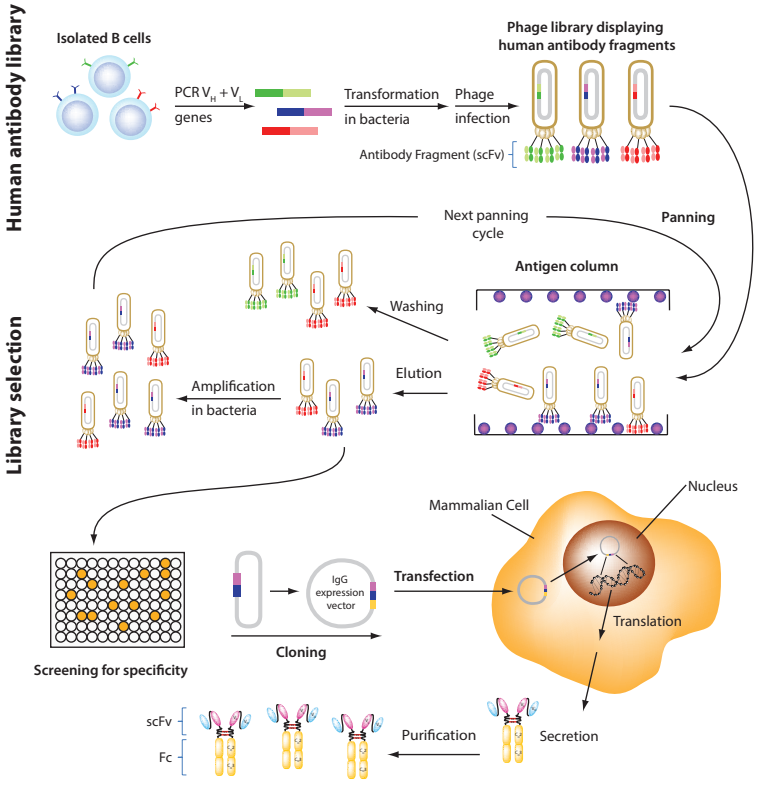
Recombinant Monoclonal Antibodies [RecMAbs™]

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 antigen-specific 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).

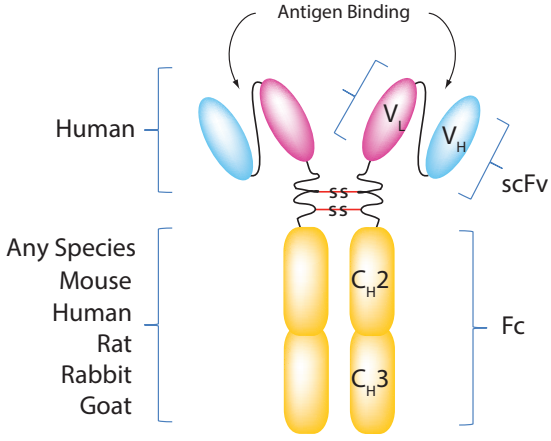
An important attribute of the RecMAbs™ phage display approach is the ability to design selection strategies to generate antibodies with customized functions (FuncAbs™), which furthermore can be classified based on activity (see frontpage) or mode of binding. For instance, it is possible to generate RecMAbs™ 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™)



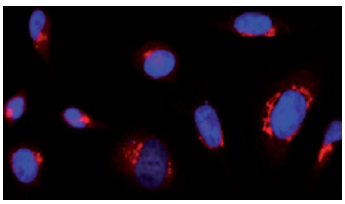
Antibodies Mode of Binding

Structure of AdipoGen RecMAbs™



Conformation-specific Recombinant Antibodies

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



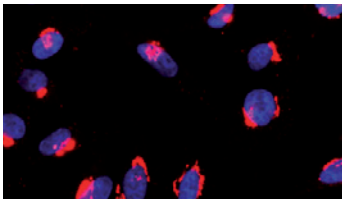
AG-27B-0006 — 100 µg

Isotype 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)



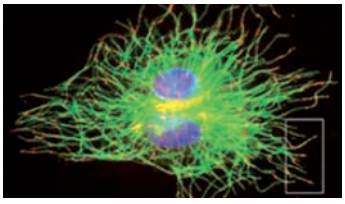
AG-27B-0004 — 100 µg
AG-27B-0004TD ATTO 488 — 100 µg

Isotype Human IgG2bλ
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 — 100 µg

Isotype 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 IgG2λ	ICC	Hu, Ms
anti-Myosin IIA (non-muscle) (heavy chain), mAb (rec.) (SF9)	AG-27B-0010	100 µg	Human IgG2λ	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 IgG2λ	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 IgG2λ	ELISA, WB	Hu, Ms, Rt
anti-IL-1R2 (mouse), mAb (rec.) (Praxy-1-1)	AG-27B-0011	100 µg	Human IgG2λ	ELISA, FACS	Ms
anti-IL-33 (mouse), mAb (rec.) (Carly-1-4)	AG-27B-0012	100 µg	Human IgG2λ	ELISA, WB	Ms
anti-PEDF (human), mAb (rec.) (Serpy-1-4)	AG-27B-0014	100 µg	Human IgG2λ	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

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

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, Δ2-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.

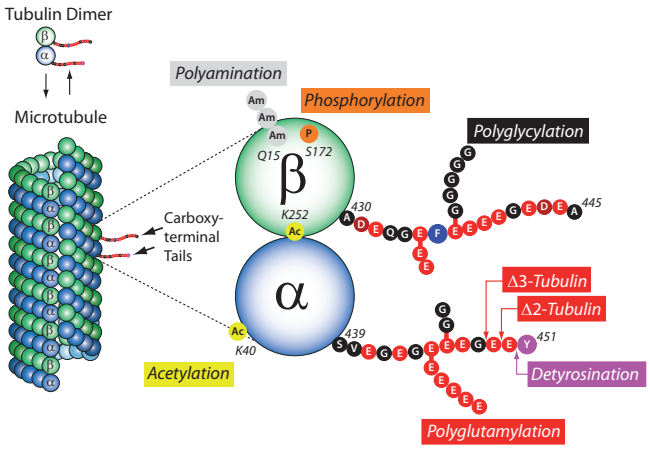

FIGURE: Tubulin PTM Overview. *Adapted from C. Janke; J. Cell. Biol.* ***206,*** *461 (2014)*

Recombinant Microtubule-target Antibodies

RecMAbs™

ANTIBODIES	PID	SIZE	ISOTYPE/SOURCE	APPLICATION	SPECIES
anti-Tubulin-GTP, mAb (rec.) (MB11) UNIQUE	AG-27B-0009	100 µg	Human IgG2λ	ICC	Hu, Ms, Rt, Dr
anti-α-Tubulin, mAb (rec.) (F2C)	AG-27B-0005	100 µg	Human IgG2λ	ICC, WB	Hu, Ms, Bv
anti-α-Tubulin, mAb (rec.) (F2C) (ATTO 488)	AG-27B-0005TD	100 µg	Human IgG2λ	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

UNIQUE

Validated Post-translational Modification-specific Antibodies

ANTIBODIES	PID	SIZE	ISOTYPE/SOURCE	APPLICATION
anti-α-Tubulin (acetylated), mAb (TEU318)	AG-20B-0068	100 µg	Mouse IgG1	ICC, WB
anti-Polyglutamylation Modification, mAb (GT335)	AG-20B-0020	100 µg	Mouse IgG1κ	EM, IHC, ICC, IP, WB
anti-Polyglutamylation Modification, mAb (GT335) (Biotin)	AG-20B-0020B	100 µg	Mouse IgG1κ	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

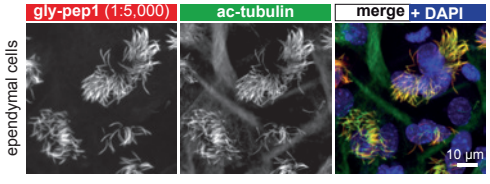

FIGURE: Immunofluorescence staining using anti-Tubulin (glycylated), pAb (Gly-pep1) (Prod. No. AG-25B-0034) of multiciliated ependymal cells and cells with primary cilia. Method: Radial glial cells isolated from newborn wildtype mice, and the MDCK cell line were serum starved to induce ciliogenesis. 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*

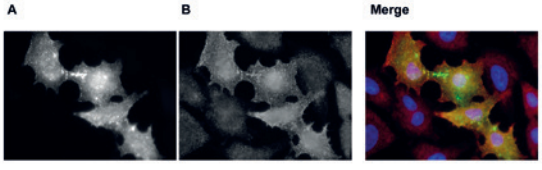

FIGURE: Palmitoylated PSD95 is detected by immunocytochemistry using anti-PSD-95 (palmitoylated), mAb (rec.) (PF11) (Prod. No AG-27B-0021). Method: HeLa cells were cotransfected with DHHC2 (palmitoylating enzyme) + PSD95-GFP (A) or DHHC2 (palmitoylating enzyme) alone (B). Cells in "B" were fixed with paraformaldehyde (3%), permeablized in PBS+ BSA 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 (ATTO488) 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*

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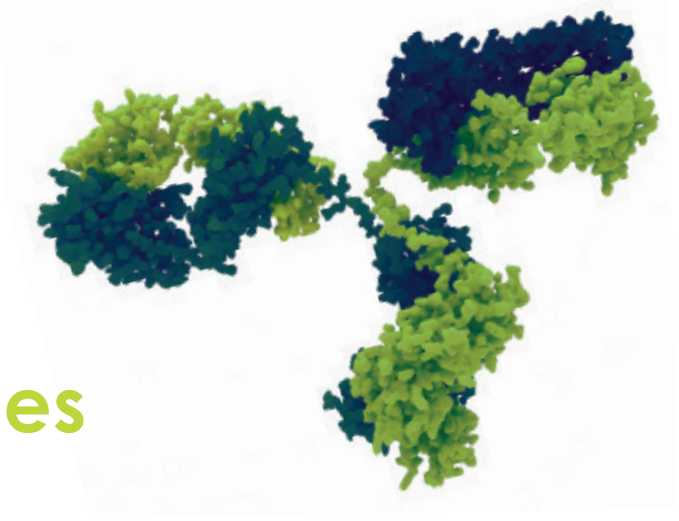

Functional Antibodies

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 µg/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 FcγRI 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 Ig 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-2Rβ (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 Ig 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 Ig 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-β1 to CD105 expressing leukemia cells.
IgM (human), mAb (UCHB1)	ANC-141	FUNC, FACS, ELISA	Delivers a costimulatory signal to B cells *in vitro*.

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-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

NEW

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

AG-20B-0075		100 µg
AG-20B-0075PF	Preservative Free	100 µg | 500 µg

Isotype Rat IgG2aκ
Application FACS, FUNC (Blocking)

Functional Application
Blocks PD-1 binding. Induces a rapid activation and proliferation of T cells at concentration of 0.25µg/2x10^5 cells.

LIT: Antibody-mediated signalling through PD-1 costimulates T cells and enhances CD28-dependent proliferation: M.L. del Rio, et al.; Eur. J. Immunol. **35,** 3545 (2005)

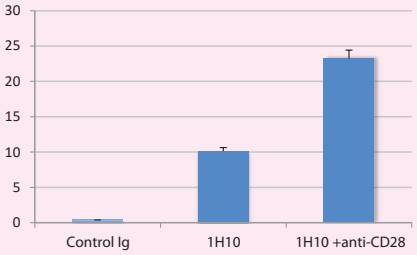

FIGURE: PD-1 receptor-induced CD4 T cell activation and proliferation by PD-1 (mouse), mAb (blocking) (1H10) (AG-20B-0075).

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 IgG2a isotype (control Ig) (0.25µg/2x10^5 cells) for 48h. Proliferation is determined by [3H]thymidine incorporation. The presence of anti-CD28 mAb increases 1H10 mAb-mediated proliferation.

GOLD STANDARDS

Validated Inflammasome Antibodies

anti-Asc, pAb (AL177)

AG-25B-0006		100 µg
AG-25B-0006PF	Preservative Free	100 µg
AG-25B-0006TS	ATTO 647N	100 µg

Isotype Rabbit
Application ICC, IHC, IP, WB, FUNC (Inhibition)

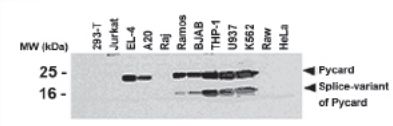

FIGURE: Western blot analysis of human and mouse cell lines using anti-Asc, pAb (AL177) (Prod. No. AG-25B-0006). Total protein extracts from various human (293-T, Jurkat, Raj, Ramos, BJAB, THP-1, U937, K562, Raw, HeLa) and mouse (EL-4, A20) cell lines were run on SDS-PAGE and Pycard detected by anti-Asc, pAb (AL177) at 1:1'000 dilution. Anti-rabbit IgG coupled horse radish peroxidase was used at 1:5'000 dilution for ECL detection.

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

AG-20B-0042		100 µg
AG-20B-0042B	Biotin	100 µg

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

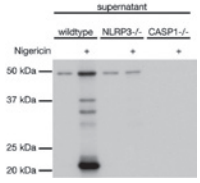

FIGURE: Mouse caspase-1 (p20) is detected by immunoblotting using anti-Caspase-1 (p20) (mouse), mAb (Casper-1) (Prod. No. AG-20B-0042).

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		100 µg

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

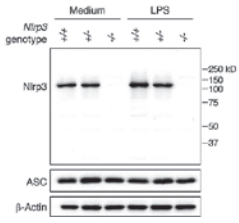

FIGURE: Mouse NLRP3 is detected in mouse macrophages using the monoclonal antibody to NLRP3 (Cryo-2) (Prod. No. AG-20B-0014).

METHOD: Cell extracts from mouse macrophages (BMDMs) WT (+/+) (lane 1), NLRP3 +/- (lane 2) or NLRP3 -/- (lane 3) with or without treatment with LPS (50ng/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.

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