

# Advanced Immune Regulation Reagents

3<sup>rd</sup> Edition

## THE SOURCE

### MultimericCD40L – A Potent B Cell Expansion Tool

MultimericCD40L™ is a high activity construct that very effectively simulates the natural membrane-assisted aggregation of CD40L. It provides a simple and equally potent alternative to CD40L + enhancer combinations. The CD40 Ligand Multi-mer mimics a T cell-dependent activation of B cells. AdipoGen Life Sciences provides unique CD40L (multimeric) proteins (mouse and human) that can be used for the activation and expansion of mouse and human B cells.

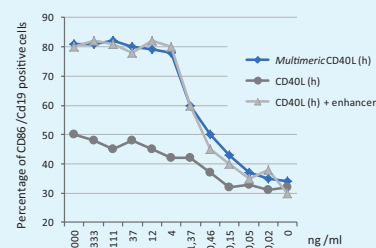
#### CD40L (human) (multimeric) (rec.)

AG-40B-0010 10 µg | 3 x 10 µg  
AG-40B-0010B **NEW** Biotin 10 µg | 3 x 10 µg

#### CD40L (mouse) (multimeric) (rec.)

AG-40B-0020 10 µg | 3 x 10 µg

**FIGURE:** CD40L (human) (multimeric) (rec.) (Prod. No. AG-40B-0010) does not need an enhancer to induce B cell activation.



## BULK

### Potent Agonistic CD40 Antibody

anti-CD40 (mouse), mAb (FGK45) (preservative free)

AG-20B-0036PF 100 µg | 500 µg | 5 mg

Activates B and NK cells *in vivo* and *in vitro*!

## NEW

### Fibrinogen-like Protein 1 (FGL1) – A Major Ligand of LAG-3

LAG-3 is a co-inhibitory immune checkpoint receptor that inhibits T cell response by binding to MHC-II. A recent study identifies Fibrinogen-like Protein 1 (FGL1), a molecule secreted by the liver and pancreas, as a major ligand for LAG-3 in both human and mouse. FGL1 binding to LAG-3 inhibits T cell response. FGL1 is upregulated in human cancers and high plasma FGL1 levels are associated with poor clinical outcome in patients treated with anti-PD1 therapy.

**LIT:** Fibrinogen-like Protein 1 Is a Major Immune Inhibitory Ligand of LAG-3: J. Wang, et al.; Cell 176, 334 (2019)

PROTEINS	PID	SIZE	SOURCE	ENDOTOXIN	SPECIES
<b>NEW</b> Fc (human):FGL1 (human) (rec.)	AG-40B-0184	10 µg   3 x 10 µg   100 µg	HEK 293 cells	<0.01EU/µg	Hu
<b>NEW</b> Fc (human):FGL1 (mouse) (rec.)	AG-40B-0185	10 µg   3 x 10 µg   100 µg	HEK 293 cells	<0.01EU/µg	Ms
<b>NEW</b> FGL1 (human) (rec.) (His)	AG-40B-0186	10 µg   3 x 10 µg	HEK 293 cells	<0.01EU/µg	Hu

## VISTA:COMP – Immunosuppressive *In Vivo* Agonist

VISTA is a new negative checkpoint regulator that potently suppresses T cell activation. Recently, it has been reported that recombinant VISTA protein needs to be **pentamerized to be active** as soluble ligand. The protein VISTA (mouse):COMP (mouse) (with the extracellular domain of mouse VISTA fused to the pentamerization domain from the cartilage oligomeric matrix protein (COMP), but not VISTA-Fc, functions as an immunosuppressive agonist *in vivo* inhibiting the proliferation of CD4<sup>+</sup> T cells.

**LIT:** VISTA:COMP - an engineered checkpoint receptor agonist that potently suppresses T cell-mediated immune responses: A. Prodeus, et al.; JCI Insight 2, e94308 (2017)

PROTEINS	PID	SIZE	SOURCE	ENDOTOXIN	SPECIES
<b>VISTA (mouse):COMP (mouse) (rec.) (His)</b>	AG-40B-0181	50 µg	HEK 293 cells	<0.01EU/µg	Ms
<b>NEW VISTA (human):COMP (mouse) (rec.) (His)</b>	AG-40B-0183	50 µg	HEK 293 cells	<0.01EU/µg	Hu

## Expansion of T Regulatory Cells (Tregs)

IL-33 has emerged as a multifunctional protein with roles in driving potent type 1 and type 2 immunity as well as facilitating profound Treg expansion *in vitro* and *in vivo*. IL-33-expanded Tregs express the IL-33 receptor ST2 and classical markers associated with Treg phenotype and suppressor function. IL-33 may potentially be used to promote/maintain organ transplant tolerance or suppress autoimmunity and has been associated with Treg-mediated wound healing. Recently, IL-33 has also been found as a critical cytokine for the expansion of brain Treg cells that potentiates neurological recovery.

**LIT:** Poor repair of skeletal muscle in aging mice reflects a defect in local, interleukin-33-dependent accumulation of regulatory T cells: W. Kuswanto, et al.; Immunity 44, 355 (2016) • Expansion of regulatory T cells in vitro and in vivo by IL-33: B.M. Matta & H.R. Turnquist; Methods Mol. Biol. 1371, 29 (2016) • Brain regulatory T cells suppress astroglia and potentiate neurological recovery: M. Ito, et al.; Nature 565, 246 (2019)

### NEW Highly Active Human IL-33 Proteins

#### IL-33 (oxidation resistant) (human) (rec.)

AG-40B-0160	Untagged	10 µg   100 µg
AG-40B-0167	His-Tag	10 µg   100 µg

**LIT:** Oxidation of the alarmin IL-33 regulates ST2-dependent inflammation: E.S. Cohen, et al.; Nat. Commun. 6, ID8327 (2015)

## Potent Th2 Immune Response Blocking Reagents

IL-33 and its receptor ST2 are involved in the Th2 immune response in allergic inflammation and parasite infection. AdipoGen Life Sciences provides powerful and specific reagents to block the IL-33/ST2 axis and the Th2 response.

#### HpARI (Alarmin Release Inhibitor) (rec.) (His)

AG-40B-0178 50 µg | 3 x 50 µg  
Natural IL-33 (human/mouse) interfering reagent from a parasite.

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

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

## Proteasome Inhibitors – The Widest Panel

**Bortezomib [PS-341] | Carfilzomib [PR-171] | Delanzomib [CEP-18770] | Epoxomicin | Ixazomib [MLN2238] | Ixazomib citrate [MLN9708] | clasto-Lactacystin β-lactone | Lactacystin | ONX 0914 | Oprozomib [ONX 0912] | PI-1840 | Salinosporamide A | VR23**

Proteasome inhibition with potent natural or synthetic small molecules has implications in a number of human diseases such as cancer, inflammation and ischemic stroke.

# Plasma Cell Survival & Proliferation Blocking Antibodies

## **BULK** anti-BAFF (mouse), mAb (blocking) (Sandy-2)

AG-20B-0063 100 µg  
AG-20B-0063PF Preservative Free 100 µg | 500 µg

**Functional Application:** **Depletion of B cells *in vivo***. Inhibition of mouse BAFF binding to BAFF-R and TAC1 (BCMA not tested); blocks BAFF activity in mice.

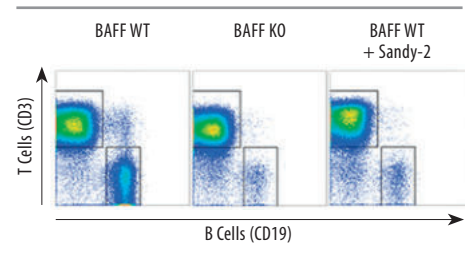
**LIT:** The B cell-stimulatory cytokines BlyS and APRIL are elevated in human periodontitis and are required for B cell-dependent bone loss in experimental murine periodontitis: T. Abe, et al.; J. Immunol. 195, 1427 (2015)

## **BULK** 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

**Functional Application:** **Depletion of plasma cells**. Inhibits binding of mouse APRIL to mouse BCMA and TAC1.

**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)



**FIGURE:** anti-BAFF (mouse), mAb (Sandy-2) (Prod. No. AG-20B-0063) 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) (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.

## Multimeric & Mutant Cytokines with Increased Activity

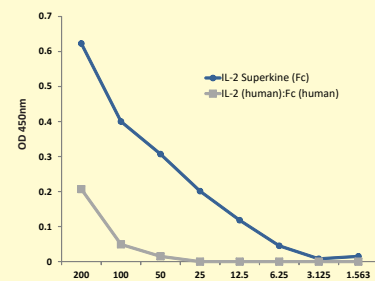
### IL-2 Superkine (Fc)

AG-40B-0111 10 µg | 3 x 10 µg

Compared to IL-2, IL-2 Superkine does not require CD25 to be active and induces superior expansion of cytotoxic CD8<sup>+</sup> T and NK cells, leading to improved antitumor responses *in vivo*. IL-2 Superkine activates only poorly T regulatory cells (Tregs), meaning less toxic effects *in vivo*.

**LIT:** Exploiting a natural conformational switch to engineer an interleukin-2 'superkine': AM. Levin, et al.; Nature 484, 529 (2012)

**FIGURE:** Binding of IL-2 Superkine (Fc) (Prod. No. AG-40B-0111) to IL-2Rβ (human) is increased 10 fold compared to IL-2 (human):Fc (human).



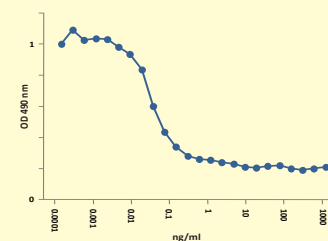
### BAFF, Soluble (human) (60-mer) (rec.) (highly active)

AG-40B-0112 10 µg | 3 x 10 µg

**Biological Activity:** Increases B cell survival/proliferation. Increases CD21/CD23 expression on B cells *in vivo*. **Activates BAFF-R, TAC1 and BCMA receptors**. Works at concentrations <20ng/ml.

**LIT:** Mutation of the BAFF furin cleavage site impairs B-cell homeostasis and antibody responses: C. Bossen, et al.; Eur. J. Immunol. 41, 787 (2011)

**FIGURE:** BAFF, Soluble (human) (60-mer) (Prod. No. AG-40B-0112) binds and activates BCMA receptor.



## UNIQUE

## Posttranslational Modification-Specific Tubulin Antibodies

ANTIBODIES	PID	SIZE	ISOTYPE/SOURCE	APPLICATION
anti-α-Tubulin (acetylated), mAb (TEU318)	AG-20B-0068	100 µg	Mouse IgG1	ICC, WB
<b>NEW</b> anti-Tubulin (glycylated), pAb (Gly-pep1)	AG-25B-0034	100 µg	Rabbit	ICC, IP, WB
anti-Tubulin-GTP, mAb (rec.) (MB11)	AG-27B-0009	100 µg	Human IgG2κ	ICC
anti-Polyglutamylation Modification, mAb (GT335)	AG-20B-0020	100 µg	Mouse IgG1κ	EM, ICC, IP, WB
anti-Polyglutamylation Modification, mAb (GT335) (Biotin)	AG-20B-0020B	100 µg	Mouse IgG1κ	ICC, IP, WB
anti-Polyglutamate chain (polyE), pAb (IN105)	AG-25B-0030	50 µg	Rabbit	ICC, WB

# Inflammasome Research Antibodies

**THE STANDARDS**

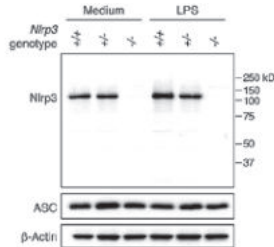
FROM THE EXPERTS &amp; VALIDATED BY EXPERT LABORATORIES!

## Unique NLRP3 Antibody

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

ICC, IHC, IP, WB

AG-20B-0014



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

#### PROTOCOLS FOR CASPER-1, CASPER-2, BALLY-1 AND CRYO-2:

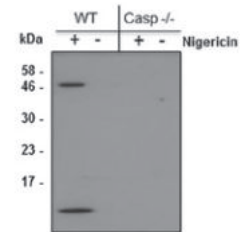
Measuring the inflammasome: O. Gross; Methods Mol. Biol. 844, 199 (2012) • Measuring NLR Oligomerization I: Size Exclusion Chromatography, Co-immunoprecipitation, and Cross-Linking: S. Khare, et al.; Methods Mol. Biol. 1417, 131 (2016)

## Detect Activated p10 & p20 Caspase-1 by WB

**anti-Caspase-1 (p10) (mouse), mAb (Casper-2) AG-20B-0044**
**anti-Caspase-1 (p20) (mouse), mAb (Casper-1) AG-20B-0042**
**anti-Caspase-1 (p20) (human), mAb (Bally-1) AG-20B-0048**

**FIGURE:** Mouse caspase-1 (p10) is detected by immunoblotting using anti-Caspase-1 (p10) (mouse), mAb (Casper-2) (Prod. No. AG-20B-0044).

**METHOD:** Caspase-1 was analyzed by Western blot in supernatants of differentiated bone marrow-derived dendritic cells (BMDCs) from wild-type and caspase-1<sup>-/-</sup> mice activated or not by 5 $\mu$ M nigericin (Prod. No. AG-CN2-0020) for 30 min. Supernatants (30 $\mu$ l) were separated by SDS-PAGE under reducing conditions, transferred to nitrocellulose and incubated with anti-Caspase-1 (p10) (mouse), mAb (Casper-2) (1 $\mu$ g/ml). Proteins were visualized by a chemiluminescence detection system.



## Key Antibodies

<b>anti-Asc [Pycard], pAb (AL177)</b>   ICC, IHC, IP, WB, FUNC	AG-25B-0006
<b>anti-AIM2 (human), mAb (3B10)</b>   ICC, WB	AG-20B-0040
<b>anti-Caspase-4/11 (p20), mAb (Flamy-1)</b>   IP, WB	AG-20B-0060
<b>anti-RIG-I, mAb (Alme-1)</b>   IHC, IP, WB	AG-20B-0009
<b>anti-ZBP1, mAb (Zippy-1)</b>   ICC, IP, WB	AG-20B-0010

**BULK**

## In vivo Inflammasome Inhibitor

**MCC950 . sodium salt (>98%)**

AG-CR1-3615

1 mg | 5 mg | 10 mg

**BULK**

## From the Manufacturer of iNKT Stimulators

 **$\alpha$ -Galactosylceramide (>96%) [KRN 7000]**

AG-CN2-0013

 250  $\mu$ g | 1 mg

**UNIQUE**

## Highly Active DLL4 Notch Ligand

**NEW** DLL4 (human):Fc (human) (rec.) (highly active mutant)

AG-40B-0176

 10  $\mu$ g | 100  $\mu$ g

Interacts with human Notch1 with &gt;20 fold increase in affinity relative to WT DLL4 (as confirmed by ELISA).

Visit [www.adipogen.com](http://www.adipogen.com) for a **Complete Panel of Notch-related Reagents!**



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