

# OPTIMIZE IMMUNOASSAY DEVELOPMENT



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Selecting

# **Reagents** for Diagnostic Immunoassays

A complete suite of antibodies and reagents for the development and optimization of accurate and sensitive immunoassays for diagnostic serological applications.

### **IMMUNOREAGENTS FOR**

# Serology and diagnostics design and production

High-quality reagents are essential to ensure accuracy, sensitivity, and reproducibility - the key requirements of a reliable diagnostic test. In this brochure we describe some of the critical considerations necessary to select the right detection reagents for your immunoassay.



# What type of assay are you developing?

Assay development depends on the sensitivity, speed, and ease of use required, along with access to equipment and how the test is to be administered.

#### **Common** serological tests are:

Bead-based assays
ELISA
Lateral Flow
Western Blot
Nephelometric/Turbidimetric
Flow Cytometry

## What format will you be using?

Immunoassays can detect a diverse array of molecules like a structural motif on a virus particle or allergen, or immunoglobulins from biological fluids of hyperimmune patients, such as serum or saliva.

#### Immunoassay formats

Assays can be set up in a variety of formats including, direct, indirect, sandwich, and competitive. Indirect and sandwich assays are the more commonly used formats. They offer a higher level of sensitivity by way of the conjugated secondary antibody amplifying the signal from the target molecule.





# Detection and capture antibodies for serological tests

#### Are other host species present in your assay?

Serological tests typically do not have species-to-species crossreactivity complications due to the nature of their design. **Table 1** suggests anti-human antibodies suitable for immunoassays where cross-reactivity from other species proteins will not be present. Pan-specific antibodies allow the measurement of total Ig of two or more Ig classes. These products will not differentiate IgG, IgM, and IgA.

Product Description	Product code
Goat Anti-Human Serum IgA, $lpha$ chain specific	109-005-011
Goat Anti-Human IgM, Fc5µ fragment specific (min X Bov Sr Prot)	109-005-129
Goat Anti-Human IgG, Fc $\gamma$ fragment specific	109-005-008
Goət Anti-Humən IgA + IgG + IgM (H+L)	109-005-064

**Table 1**: Anti-Human antibodies suitable for the detection of human immunoglobulinsin the absence of antibodies from other species.



# Cross-absorption for the ultimate in specific detection

In immunotechniques that require antibodies from multiple species in the assay system, crossadsorbed secondary antibodies are recommended to avoid detection of Igs that are not the intended target, minimizing non-specific signal.

Cross-adsorbed secondary antibodies are purified to prevent species cross-reactivity that occurs when Ig from one species recognizes a homologous epitope of an Ig from another species. Cross-adsorption removes antibodies that happen to recognize shared epitopes on other proteins, removing the likelihood of non-specific detection. For applications in which species cross-reactivity is not a concern, the use of cross-adsorbed antibodies is optional.

# Clonality - Monoclonal or Polyclonal Antibodies for your assay?

Monoclonal antibodies should be screened to identify those that perform well under assay conditions. Polyclonal antibodies are simple to produce in large quantities and can achieve greater assay sensitivity. Being a mixture of Igs, each recognizing a different epitope on the antigen simultaneously, they allow for more reporter molecules to be deposited, increasing signal.

**Monoclonal antibodies** 







#### **Polyclonal antibodies**

Polyclonal antibodies

Patient antibody

Fluorophore

# **Reagents in a wide** range of host species

Jackson ImmunoResearch produces antibodies in a wide range of host species, with goat being a common choice for products moving from research and development to commercialization. Larger host animals facilitate the production of greater volumes of raw material, accommodating consistent batch quality even for large lot sizes.

Our goal is to ensure that customers can select the best products for their large-scale assau and kit development needs while offering competitive cost-per-assay pricing. We also manufacture Anti-Human antibodies with strong affinity and avidity in mice, rabbits, alpacas, and donkeys.





### Selection of the host species for a secondary antibody involves many considerations, including:





#### A. Both Goat and Rabbit antibodies displayed almost identical limits of detection and signal strength in this assay.

Plates were coated with AffiniPure Goat Anti-Human IgG, Fcy fragment specific (109-005-008) or AffiniPure Rabbit Anti-Human IgG, Fcy fragment specific (390-005-008) and blocked with BSA (001-000-162). ChromPure Human IgG, whole molecule (009-000-003) was titrated across plate and washed. IgG was detected with HRP AffiniPure Goat Anti-Human IgG, Fcy fragment specific (109-035-008) or HRP AffiniPure Rabbit Anti-Human IgG, Fcγ fragment specific (309-035-008). Goat and Rabbit antibodies displayed almost identical limits of detection and signal strength in this assay.

displayed similar limits of detection and have strong signal to noise. Plates were coated with AffiniPure GoatAnti-Human IgM, Fc5µ fragment specific (109-005-129) or AffiniPure Rabbit Anti-Human IgM, Fc5µ fragment specific (309-005-095) and blocked with BSA (001-000-162). ChromPure Human IgM (myeloma), whole molecule (009-000-012) was titrated across plate and washed. IqM was detected with HRP AffiniPure Goat Anti-Human IgM, Fc5µ fragment specific (109-035-129) or Peroxidase AffiniPure Rabbit Anti-Human IgM, Fc5µ fragment specific (309-035-095). Goat and Rabbit antibodies displayed similar limits of detection with strong signal to noise.

# **Does host** species matter?

To explore the influence of host species on antibody performance, we compared two popular host species, Rabbit and Goat, to identify any difference in the detection of human IgG, IgM, and IgA by ELISA, to demonstrate that the specificity and sensitivity of JIR Goat Anti-Human and Rabbit Anti-Human antibodies are comparable for detecting IgG, IgM or IgA.

# B. Both Goat and Rabbit antibodies



#### C. Both Goat and Rabbit antibodies displayed similar limits of detection and high signal to noise.

Plates were coated with AffiniPure Goat Anti-Human Serum IgA, a chain specific (109-005-011) and blockedwith BSA (001-000-162). ChromPure Human Serum IgA, whole molecule (009-000-011) was titrated across plate and washed. IgA was detected with HRP AffiniPure Goat Anti-Human Serum IgA, a chain specific (109-035-011) or HRP AffiniPure Rabbit Anti-Human Serum IgA, a chain specific (309-035-011).

# **Veterinary testing**

Not only do human patients need accurate and sensitive diagnostic and surveillance testing, but with zoonotic diseases disrupting people's lives across the world, testing of animals is essential. We manufacture antibodies with specificity against a wide range of other species, from farm animals such as pigs, cattle, sheep, and poultry, through to companion animals such as cats and dogs.

For more information please speak with a JIR representative: sales@jacksonimmuno.com | (800) 367-5296





# Detecting specific classes of immunoglobulin

When immunoglobulin specificity needs are paramount you can trust JIR secondary antibodies to differentiate with exquisite accuracy. **Figure 3** shows the specificity of JIR Anti-Human IgG (109-035-008) and JIR Anti-Human IgM (109-035-129) for their intended target Ig in the presence of other immunoglobulins.



**Figure 3:** SARS-CoV-2 Serology ELISA: The SARS-CoV-2 serology ELISA was carried out following the procedure described in Amanat et al. (2020). Briefly, ELISA plates were coated with SARS-CoV-2 S1 protein (Genscript Z03485) and plates were blocked with 1% Bovine Serum Albumin (IgG-Free, Protease-Free) (001-000-162) in PBST. Serial dilutions of Human IgG SARS-CoV-2 Spike S1 Antibody (Genscript A02038) and Human IgM SARS-CoV-2 Spike S1 Antibody (Genscript A02046) were added to successive wells and the non-binding fraction was washed off. Captured IgG and IgM were detected with Peroxidase AffiniPure Goat Anti-Human IgG, Fcγ fragment specific (109-035-008) and Peroxidase AffiniPure Goat Anti-Human IgM, Fc5μ fragment specific (109-035-129), respectively, diluted 1:20,000 in PBST.

\*Serological assay conditions based on Mt. Sinai EUA April 15, 2020 (Amanat et al., 2020)



# Reporter molecules and conjugates

Reporter molecules commonly used in immunoassays include colloidal gold, reporter enzymes and fluorescent dyes. Their selection depends on assay objectives and readout method.

Reporter Enzymes and Amplification Molecules		
Horse Radish Peroxidase		
Streptavidin		
Alkaline Phosphatase		
Biotin		
ImmunoGold		
40nm		
18nm		

12nm 6nm 4nm

Fluorescent Proteins	Excitation Peak	Emission Peak
Aminomethylcoumarin, AMCA	350 nm	450 nm
R-Phycoerythrin, R-PE	many, 488 nm	580 nm
Allophycocyanin, APC	məny, 650 nm	660 nm
Peridinin-Chlorophyll-Protein, PerCP	many, 488 nm	675 nm

Fluorescent dyes	Excitation Peak	Emission Peak
DyLight™ 405	400 nm	421 nm
Brilliant Violet 421™	407 nm	421 nm
Brilliant Violet 480™	436 nm	478 nm
Alexa Fluor® 488	493 nm	519 nm
Fluorescein, FITC/DTAF	492 nm	520 nm
Alexa Fluor® 555	552 nm	572 nm
Indocarbocyanine, Cy™3	550 nm	570 nm
Rhodəmine Red™-X, RRX	570 nm	590 nm
Alexa Fluor® 568	577 nm	602 nm
Alexa Fluor® 594	591 nm	614 nm
Alexa Fluor® 647	651 nm	667 nm
Indodicarbocyanine, Cy™5	650 nm	670 nm
Alexa Fluor® 680	684 nm	702 nm
Alexa Fluor® 790	792 nm	803 nm

## 40 nm Colloidal Gold Conjugates

40 nm Colloidal Gold is a popular reporter molecule for lateral flow immunoassays (LFIAs), producing intense color and strong signals, making result readout easy. The intensity of the color generated is due to the plasmon absorbance and light scattering of the gold's electron shell\*, and the small particles' ability to pack at high density on the test and control lines. Jackson ImmunoResearch 40 nm gold conjugates are consistent and reliable, with the expected sensitivity of our antibodies and availability in a wide range of formats and specificities perfect for assay production.

40 nm conjugate	Product code
Goat Anti-Human IgG, Fc fragment specific	109-405-008
Goat Anti-Human IgG, Fcγ fragment specific (min X Bov, Ms, Rb Sr Prot)	109-405-170
Goat Anti-Human IgM, Fc5µ fragment specific (min X Bov Sr Prot)	109-405-129
Mouse IgG Fraction Monoclonal Anti-Biotin	200-402-211
ChromPure Chicken IgY (IgG)	003-400-003
Streptavidin	016-400-084

#### Fluorescent Dye Conjugates

Fluorescent dyes may be used for a wide range of immunoassays including flow cytometry based tests. Fluorescein and Cyanine dyes are popular choices.

Conjugate	Product Description	Product cod
Fluorescein (FITC)	Goət Anti-Humən IgG, Fcγ fragment specific (min X Bov, Ms Sr Prot)	109-095-19
Fluorescein (FITC)	Goat Anti-Human IgM, Fc5µ fragment specific	109-095-04
Cy™2	Donkey Anti-Chicken IgY (IgG) (H+L)	703-225-15
Fluorescein (FITC)	ChromPure Chicken IgY (IgG), whole molecule	003-090-00



# Complementary products for assay optimization



# **Signal** boosting strategies for effective tests - Blocking

Blocking using BSA or normal serum can improve result accuracy, and reduce false positives from non-specific interactions leading to background signal. Best results are obtained with diluted normal serum from the same host as the labelled antibody, used as a separate incubation step before addition of the primary antibody. Jackson ImmunoResearch BSA is IgG-free and protease-free. It does not contain contaminating IgG, which alleviates common immunoassay problems associated with many commercial high purity preparations of BSA.



#### Anti-Chicken Antibodies for LFIAs

Capture antibodies directed against target molecules and the conjugated secondary antibodies used to detect them are typically raised in mammalian hosts. Control reagents run alongside these Igs in an assay and must not interfere with any reagents that contribute to the test result. The inherently low reactivity of anti-mammalian IgG to chicken Igs makes IgY a particularly useful control protein. Similarly, the reduced binding of anti-chicken (IgY) antibodies to mammalian IgG reduces the chance of interaction between reagents which may cause background. ChromPure IgY and Anti-Chicken antibodies are available in a wide range of conjugates including 40 nm Gold and FITC.



#### Antibodies as Control Reagents

The choice of control reagents used in an immunoassay is important. Antibodies are used as controls to either confirm that a test was performed correctly, or to troubleshoot performance. Isotype controls are antibodies which match the host species and class of antibodies used in the experiment but are not directed against the antigen of interest. They are used as negative control to estimate the non-specific binding of an antibody and should be conjugated with the same reporter molecule as the specific antibody. ChromPure<sup>™</sup> proteins are purified from the serum of non-immunized animals and can be used as experimental controls.



### Streptavidin for optimum assay sensitivity

Commonly employed in assays where optimal sensitivity is required, the binding characteristics of streptavidin enables signal amplification when used in combination with biotinylated reagents such as Biotin-SP-conjugated affinitypurified secondary antibodies and ChromPure™ proteins, as well as with any biotinylated primary or secondary antibodies, or oligonucleotides. Jackson ImmunoResearch offers a comprehensive list of fluorophores and enzymes conjugated to streptavidin for use in enzyme immunoassays, immunohistochemistry, flow cytometry, in situ hybridization, and immunoblotting procedures.

# **NEW** from Jackson **ImmunoResearch**

## Human Serum, Pre-SARS-CoV-2 Sourced before August 2019

Human serum collected before August 2019 is suitable for use as negative control material in immunoassays such as ELISAs requiring naive serum obtained prior to the outbreak of Covid 19. Serum sourced before August 2019 was tested for SARS-CoV-2 Spike S1 Receptor Binding Domain (RBD) antibodies as shown in panel on right.



#### Anti-Human IgE Antibodies

Specific for Human IgE antibodies, our new Mouse Monoclonal Anti-Human IgE Antibodies complement our existing Anti-Human IgG, IgM, and IgA antibodies and are the latest addition to our suite of products suitable for diagnostics research and development. We offer two clones to enable flexible assay design. Conjugates are available in a select range of reporter molecules, including Alkaline Phosphatase and Biotin, for excellent assay sensitivity.





# Antibody supply from Jackson ImmunoResearch

# Our focus and expertise

Over 40 years of experience in a single, highly specialized field; secondary antibody manufacture, conjugation, and supply.



Immunodiagnostics remain at the forefront of disease surveillance, prevention, and treatment. We understand the need for reliability, quality, and availability to meet the challenges of current times. We are certified to ISO 9001:2015 for the development and manufacture of immunological reagents.

The combination of our experience, focus on secondary antibodies, and commitment to the highest quality standards ensures that customers can be completely confident of product quality and consistency. Standard inventory immunoreagents can be guickly scaled up to bulk quantities, or produced to custom specifications upon request.

Commitment to the life-science community

Long-term customer relationships as an established and

Fast, efficient customer service and technical support

Commitment to consistent high standards across research and development, production and distribution

Guaranteed product quality, long term product consistency,

#### **Custom** services for diagnostics manufacture



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# **Specializing** in Secondary Antibodies and Conjugates



# Questions?

Contact us at sales@jacksonimmuno.com

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