

Atto Antibodies

Next generation antibodies

Catalogue

Atto514

Superior photostability

Atto5

Strong flu

F(ab)

IgG

goat-anti mouse IgG

Atto647N

High quantum yield

Atto514

Superior photostability

Atto700

High affinity

goat-anti mouse IgM

Atto390

Atto550

Atto532

Minimal Stokes shift

Atto594

Atto488

Strong fluorescence

goat-anti rabbit IgG

IgM

F(ab)₂

SATISFACTION + GUARANTEE



Welcome to Hypermol...

... and welcome to the interactive catalogue about Atto secondary antibodies manufactured for you by us.

What's new? More information, more products, and now you can visit all products online by clicking on the Catalogue number. Please use the service section to contact us for any requests, or to get your individual quote.

Uses and advantages of Atto Secondary Antibodies



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Fluorescent Antibody Kits

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It is always the quality that matters.

ANTIGEN-TARGETING

Antibodies are proteins produced by the immune system of the host, in response to exogenous molecules entering the body (“immune response”).

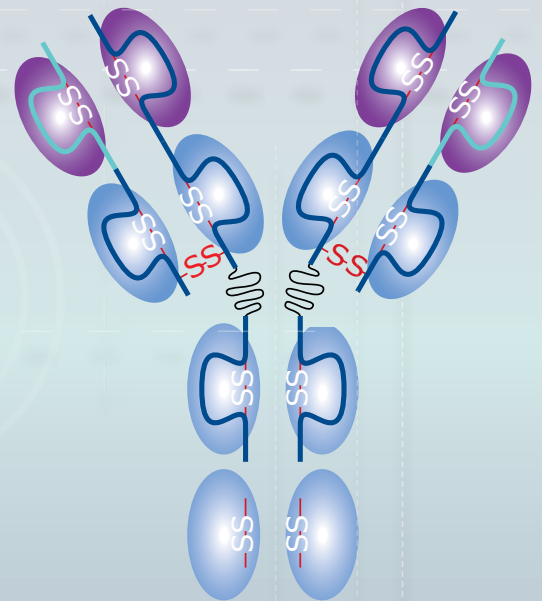
These exogenous molecules - termed antigens - and their molecular recognition by the immune system results in the selective production of antibodies with highly specific antigen recognition sites.

Antibodies are produced by B lymphocytes, which circulate throughout the blood- & lymphsystem, where they can bind specifically to an antigen which in turn results in the clearance of the antigen from the host.

This immune response system with its capability to produce highly specific antigen binding is used to produce antibodies for the detection of molecules (proteins, lipids etc.) which are of interest in research.

The use of these highly specific molecular recognition tools allows researchers to target antigens of interest e.g. of cellular structures and to investigate molecular interactions of isolated molecules in high resolution microscopic techniques, to name only a few of numerous applications.

The basis of it all is provided by the specific features of antibodies which make them particularly conducive to development as probes. For example, except for the antigen binding region, antibody classes have a rather uniform protein structure enabling reproducible purification, labeling and detection.



Atto514
Superior photostability

Atto594

Strong fluorescence

Atto700

Atto488

goat-anti rabbit IgG

High affinity

goat-anti mouse IgM

Atto390

Atto550

Atto647N

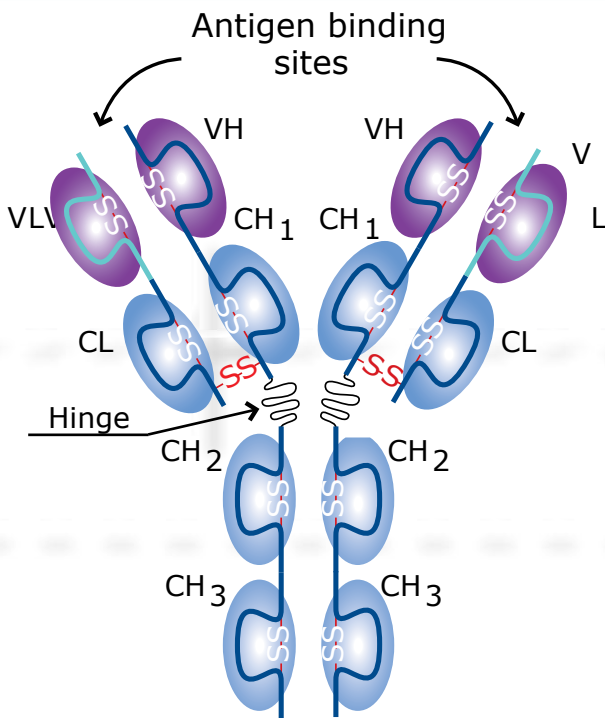
High quantum yield

Atto53

Minimal Sto

ANTIBODY STRUCTURE

Structure of Immunoglobulins



Antibodies are glycoproteins, composed of one or several units. Each unit contains four polypeptide chains: two identical heavy chains (H) and two identical light chains (L). The N-terminal ends of heavy and light chains are termed variable (V) regions, due to the variations in the amino acid sequence responsible for the high specificity of antigen recognition. Immunoglobulins also possess relatively constant (C) regions.

Each light chain consists of a single variable domain (VL) and one constant domain (CL). The heavy chains consist of a variable domain (VH), and in addition three constant domains CH1, CH2 and CH3.

Immunoglobulin G fragments

An IgG digested by papain yields two F(ab) fragments and the Fc fragment, which is subsequently removed by ion exchange chromatography (IEX).

In contrast, F(ab')₂ fragment antibodies are generated by limited cleavage of whole IgGs with pepsin, which removes most of the Fc region while leaving intact the area around the hinge region.

F(ab')₂ fragments have two antigen-binding F(ab) portions linked together by disulfide bridges, and are therefore termed divalent with an apparent molecular weight of ~85kDa according in SDS-PAGE. These antibodies are very useful for double labeling experiments, when primary antibodies of the same host species are used. Another great advantage is that they are very small and thus easily penetrate structures which are inaccessible for whole IgGs or even F(ab')₂ fragment antibodies.

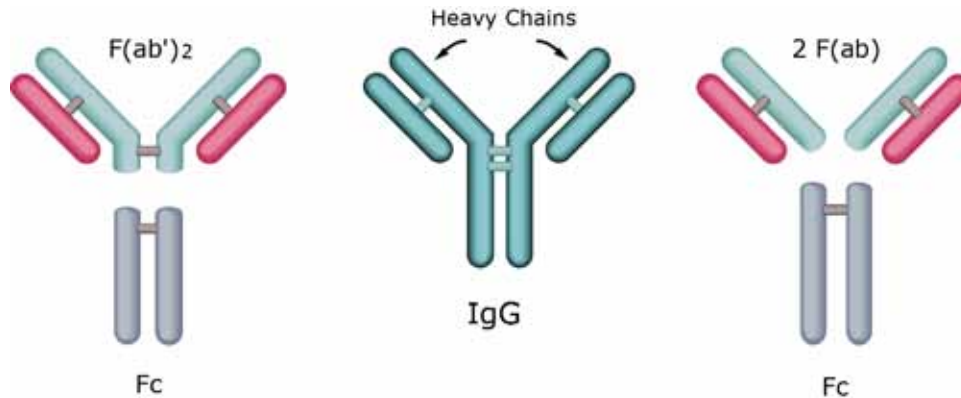
DID YOU KNOW WHY...

... our antibodies have a very high affinity!

Excellent goat sera, highly stringent affinity purification, a subsequent chain of processing and a proprietary method of highly preserving freeze-drying, make this success. The use of selected, high-quality sera makes most types of pre-absorption redundant and allows to offer best priced antibodies conjugated with the top brand Atto dyes.

WHOLE OR FRAGMENT?

When to use what



F(ab')₂ fragment secondary antibodies - divalent fragments

F(ab')₂ fragment antibodies lack most of the antibodies Fc region. Hence F(ab')₂ fragment antibodies are smaller than whole IgG antibodies and can penetrate tissues easier. This is clearly an advantage in e.g. immunocytochemistry, where F(ab)₂ fragment antibodies are frequently used.

Since IgG F(ab')₂ fragment antibodies react with light-chains, these antibodies do not only bind to IgG. Other immunoglobulins sharing the same light-chain as IgG are also recognized. F(ab')₂ fragment antibodies are used in double labeling experiments or when cells or tissues possess Fc receptors.



F(ab) fragment secondary antibodies - monovalent fragments

Compared to whole IgG and F(ab')₂ fragment antibodies, F(ab) fragment antibodies are monovalent, since they have - by definition - a single antigen binding site. F(ab) fragments of IgGs produced in goat are small (~36kDa) and contain the region responsible for antigen binding. F(ab) fragments are monovalent and the Fc region has been removed after limited enzymatic cleavage with papain (Fc fragments lack all light-chains and the upper part of the heavy-chains).

FLUORESCENT ANTIBODY KIT

What is fluorescence?

Fluorescence is the re-emission of light upon excitation of a compound. These compounds are called fluorochromes or fluorophores and have found a wide range of applications in molecular sciences. When a fluorochrome is excited by absorption of a photon, it is converted to a meta-stable state. Most important, these non-radiative transitions have to be relatively slow, in such a way that the radiative transition from excited to ground state can be successfully completed. Fluorochromes are deactivated by emission of photons, a phenomenon termed: fluorescence.

Main characteristics of fluorochromes

- Fluorescence Quantum yield (η_{fl})

is the quotient of the number of emitted (n_{fl}) and the number of absorbed photons (n_{abs}) expressed by: $\eta_{fl} = n_{fl} / n_{abs}$. It describes the efficiency of the energy transferred from absorbed photons to emitted fluorescence. The fluorescence quantum yield can not exceed 100%.

A high quantum yield is desired for fluorescent probes!

- Fluorescence Decay Time (τ_{fl})

the time interval, after which the number of molecules (n_1) in the excited state has decreased to $1/e$ (~ 0.368 or $\sim 36.8\%$) of the original amount, is called fluorescence decay time (τ_{fl}). Typical values for τ_{fl} are in the range of nanoseconds.

Background: the emission of a photon by a fluorochrome is a statistical process and the time an excited molecule stays in the excited state is in turn also statistical. Nevertheless, in a batch of identical fluorochromes, the observed decay statistics becomes well-defined. In the most simple case, the number of molecules in the excited state decreases exponentially after excitation.

The fluorescence decay time is a very important property of a fluorochrome. It is so characteristic that it even permits the identification of a fluorochrome.

Both, the fluorescence decay time and the fluorescence quantum yield are not a fixed quantities. They rather depend on the environment (solvent, temperature etc.) and are furthermore not independent quantities. They are linked by the relation $\tau_{fl} = \tau_0 \times \eta_{fl}$, τ_0 is the „natural decay time“, where the quantum yield is assumed to be 100%, defined by the complete absence of non-radiative deactivation.

- Stokes shift

is the difference between the maximum excitation and maximum emission wavelengths of the same electronic transition. For manufacturing Atto-secondary antibodies, we use those with a minimal stoke shift.

Upon absorption of a photon, a fluorochrome enters an excited state. One way for the system to relax or better, to deactivate, is to emit a photon, thus losing its energy. Most dyes deactivate by converting the absorbed energy into heat. When the emitted photon has less energy than the absorbed photon, this energy difference is the Stokes shift.

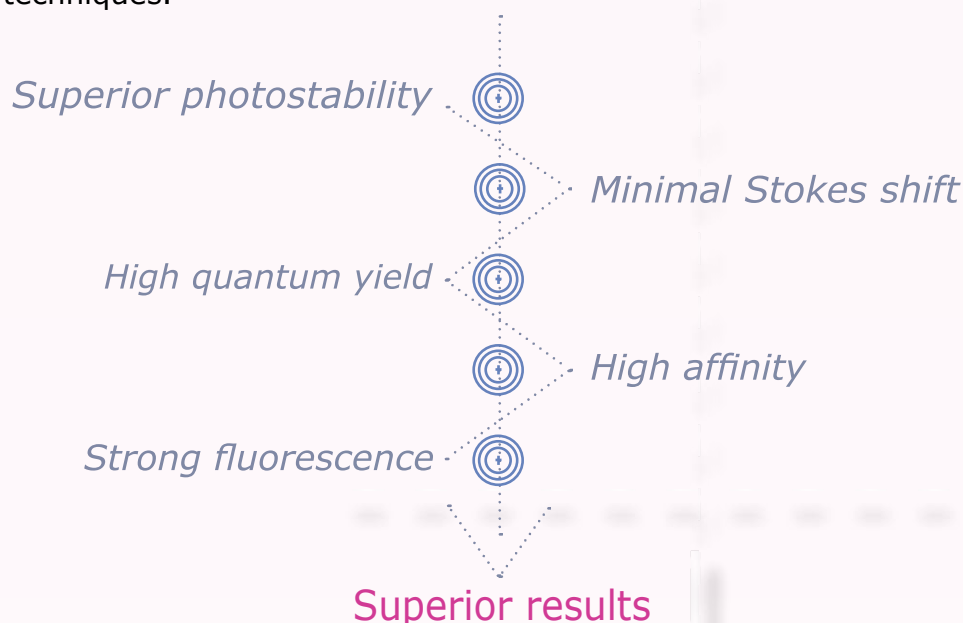


FLUORESCENT ANTIBODY KIT

Highly specific Atto secondary antibodies
- strong & long lasting fluorescence!

The quality of secondary antibodies is essential in producing superior results in immunofluorescence. Hypermol offers „Fluorescent Antibody Kits“ with secondary antibodies conjugated to the novel, high quality Atto dyes for detecting primary antibodies.

Atto-labeled antibodies belong to the new generation of fluorescent antibodies characterized by their exceptional fluorescence intensity and superior photostability. For this reason they are suited for all kinds of immunofluorescence microscopy and especially for high- and super-resolution techniques.



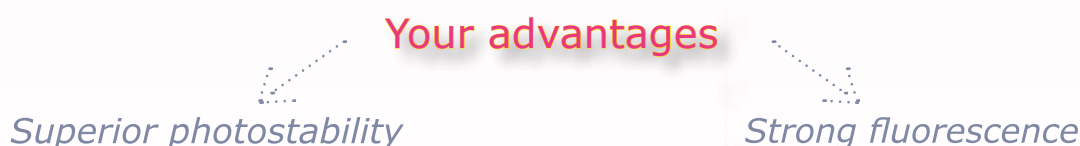
DID YOU KNOW?

Atto Antibodies are perfectly suited for epifluorescence microscopy !

The extremely high photostability makes Atto Antibodies the perfect choice for EFM. You already experienced common dyes to fade more or less rapidly during inspection. Thus epifluorescence becomes time consuming, inefficient and sometimes frustrating.

Try the next generation Atto secondary antibodies and we refund the purchase price, if our promise does not come true.

Atto-fluorescence is very stable and allows to completely inspect & image your samples.



Anti-Mouse IgG Atto produced in goat

High Sensitive Immunofluorescence Detection of Monoclonal Primary Antibodies.

The quality of secondary antibodies is essential in producing superior results in immunofluorescence detection. The affinity purified goat-anti mouse IgGs recognize with high specificity the H+L chain. Conjugated to Atto dyes known for superior features compared to common dyes, the antibodies are suited to serve highest demands in any type of fluorescence microscopy.

Fluorescent antibody kits are supplied with an epitope mapped monoclonal anti-actin for double staining or reference (1:100-1:300).

Affinity purified anti-mouse IgGs (1:500-1:1500) produced in goat are quality control tested by UV-VIS spectroscopy and immunocytochemistry.

1.0mg
each IgG-Atto



ORDERING INFORMATION

Product	Kit Contents	Cat. #
Anti-Mouse IgG Atto390 Emission maximum: 479nm Immunofluorescence:1:500-1500 Degree of labelling: 2-9	1mg (2mg/ml) goat-anti mouse IgG (H+L) Atto390;* 50µg monoclonal anti-actin (epitope mapped);* Product documentation; Certificate of analysis; * in 50% glycerol, 0.01 M sodium phosphate, 0.1 M sodium chloride, 10 mM sodium azide, pH 7.4.	2101-1MG
Anti-Mouse IgG Atto488 Emission maximum: 523nm Immunofluorescence:1:500-1500 Degree of labelling: 2-9	1mg (2mg/ml) goat-anti mouse IgG (H+L) Atto488;* 50µg monoclonal anti-actin (epitope mapped);* Product documentation; Certificate of analysis; * in 50% glycerol, 0.01 M sodium phosphate, 0.1 M sodium chloride, 10 mM sodium azide, pH 7.4.	2102-1MG
Anti-Mouse IgG Atto514 Emission maximum: 533nm Immunofluorescence:1:500-1500 Degree of labelling: 2-9	1mg (2mg/ml) goat-anti mouse IgG (H+L) Atto514;* 50µg monoclonal anti-actin (epitope mapped);* Product documentation; Certificate of analysis; * in 50% glycerol, 0.01 M sodium phosphate, 0.1 M sodium chloride, 10 mM sodium azide, pH 7.4.	2103-1MG
Anti-Mouse IgG Atto532 Emission maximum: 553nm Immunofluorescence:1:500-1500 Degree of labelling: 2-9	1mg (2mg/ml) goat-anti mouse IgG (H+L) Atto532;* 50µg monoclonal anti-actin (epitope mapped);* Product documentation; Certificate of analysis; * in 50% glycerol, 0.01 M sodium phosphate, 0.1 M sodium chloride, 10 mM sodium azide, pH 7.4.	2104-1MG

Anti-Mouse IgG Atto produced in goat

High Sensitive Immunofluorescence Detection
of Monoclonal Primary Antibodies.



ORDERING INFORMATION

Product	Kit Contents	Cat. #
Anti-Mouse IgG Atto550 Emission maximum: 576nm Immunofluorescence:1:500-1500 Degree of labelling: 2-9	1mg (2mg/ml) goat-anti mouse IgG (H+L) Atto550;* 50µg monoclonal anti-actin (epitope mapped);* Product documentation; Certificate of analysis; * in 50% glycerol, 0.01 M sodium phosphate, 0.1 M sodium chloride, 10 mM sodium azide, pH 7.4.	2105-1MG
Anti-Mouse IgG Atto565 Emission maximum: 592nm Immunofluorescence:1:500-1500 Degree of labelling: 2-9	1mg (2mg/ml) goat-anti mouse IgG (H+L) Atto565;* 50µg monoclonal anti-actin (epitope mapped);* Product documentation; Certificate of analysis; * in 50% glycerol, 0.01 M sodium phosphate, 0.1 M sodium chloride, 10 mM sodium azide, pH 7.4.	2107-1MG
Anti-Mouse IgG Atto594 Emission maximum: 627nm Immunofluorescence:1:500-1500 Degree of labelling: 2-9	1mg (2mg/ml) goat-anti mouse IgG (H+L) Atto594;* 50µg monoclonal anti-actin (epitope mapped);* Product documentation; Certificate of analysis; * in 50% glycerol, 0.01 M sodium phosphate, 0.1 M sodium chloride, 10 mM sodium azide, pH 7.4.	2106-1MG
Anti-Mouse IgG Atto647N Emission maximum: 669nm Immunofluorescence:1:500-1500 Degree of labelling: 2-9	1mg (2mg/ml) goat-anti mouse IgG (H+L) Atto647N;* 50µg monoclonal anti-actin (epitope mapped);* Product documentation; Certificate of analysis; * in 50% glycerol, 0.01 M sodium phosphate, 0.1 M sodium chloride, 10 mM sodium azide, pH 7.4.	2108-1MG
Anti-Mouse IgG Atto700 Emission maximum: 719nm Immunofluorescence:1:500-1500 Degree of labelling: 2-9	1mg (2mg/ml) goat-anti mouse IgG (H+L) Atto700;* 50µg monoclonal anti-actin (epitope mapped);* Product documentation; Certificate of analysis; * in 50% glycerol, 0.01 M sodium phosphate, 0.1 M sodium chloride, 10 mM sodium azide, pH 7.4.	2110-1MG
Anti-Mouse IgG Atto740 Emission maximum: 764nm Immunofluorescence:1:500-1500 Degree of labelling: 2-9	1mg (2mg/ml) goat-anti mouse IgG (H+L) Atto740;* 50µg monoclonal anti-actin (epitope mapped);* Product documentation; Certificate of analysis; * in 50% glycerol, 0.01 M sodium phosphate, 0.1 M sodium chloride, 10 mM sodium azide, pH 7.4.	2111-1MG

Anti-Mouse IgG Atto produced in goat



Anti-Mouse IgG F(ab')₂ Atto produced in goat

For Double Staining or Less Accessible Structures

0.5mg
each F(ab')₂-Atto

F(ab')₂ fragments are generated by limited cleavage with pepsin from affinity purified goat-anti mouse IgGs, that recognize with high specificity the H+L chain. Conjugated to Atto dyes - known for superior features compared to common dyes -, F(ab')₂ fragments are suited to serve highest demands using any type of fluorescence microscopy.

Anti-Mouse IgG (H+L) F(ab')₂ are used in dilutions 1:300-1:1000, which is suitable for most applications.

F(ab')₂ fragments are quality control tested by UV-VIS spectroscopy and immunocytochemistry.



ORDERING INFORMATION

Product	Kit Contents	Cat. #
Anti-Mouse IgG F(ab')₂ -Atto488 Emission maximum: 523nm Immunofluorescence:1:300-1000 Degree of labelling: 2-5	0.5mg (2mg/ml) anti-mouse F(ab') ₂ IgG (H+L) Atto488;* Product documentation; * in 50% glycerol, 0.01 M sodium phosphate, 0.1 M sodium chloride, 10 mM sodium azide, pH 7.4.	2402-0.5MG
Anti-Mouse IgG F(ab')₂-Atto565 Emission maximum: 592nm Immunofluorescence:1:300-1000 Degree of labelling: 2-5	0.5mg (2mg/ml) anti-mouse F(ab') ₂ IgG (H+L) Atto565;* Product documentation; * in 50% glycerol, 0.01 M sodium phosphate, 0.1 M sodium chloride, 10 mM sodium azide, pH 7.4.	2407-0.5MG
Anti-Mouse IgG F(ab')₂-Atto594 Emission maximum: 627nm Immunofluorescence:1:300-1000 Degree of labelling: 2-5	0.5mg (2mg/ml) anti-mouse F(ab') ₂ IgG (H+L) Atto594* Product documentation; * in 50% glycerol, 0.01 M sodium phosphate, 0.1 M sodium chloride, 10 mM sodium azide, pH 7.4.	2416-0.5MG
Anti-Mouse IgG F(ab')₂-Atto647N Emission maximum: 669nm Immunofluorescence:1:300-1000 Degree of labelling: 2-9	0.5mg (2mg/ml) goat-anti mouse IgG (H+L) Atto647N;* Product documentation; * in 50% glycerol, 0.01 M sodium phosphate, 0.1 M sodium chloride, 10 mM sodium azide, pH 7.4.	2418-0.5MG
Anti-Mouse IgG F(ab')₂-Atto700 Emission maximum: 719nm Immunofluorescence:1:300-1000 Degree of labelling: 2-5	0.5mg (2mg/ml) anti-mouse F(ab') ₂ IgG (H+L) Atto700* Product documentation; * in 50% glycerol, 0.01 M sodium phosphate, 0.1 M sodium chloride, 10 mM sodium azide, pH 7.4.	2411-0.5MG
Anti-Mouse IgG F(ab')₂-Atto740 Emission maximum: 764nm Immunofluorescence:1:300-1000 Degree of labelling: 2-5	0.5mg (2mg/ml) anti-mouse F(ab') ₂ IgG (H+L) Atto740;* Product documentation; * in 50% glycerol, 0.01 M sodium phosphate, 0.1 M sodium chloride, 10 mM sodium azide, pH 7.4.	2409-0.5MG



Anti-Mouse IgG F(ab) Atto produced in goat

For Double Staining or Inaccessible Structures

F(ab) fragments are generated by limited cleavage with papain from affinity purified goat-anti mouse IgGs, that recognize with high specificity the H+L chain. Conjugated to Atto dyes - known for superior features compared to common dyes -, F(ab) fragments are suited to serve highest demands using any type of fluorescence microscopy.

Anti-Mouse IgG (H+L) F(ab) are used in dilutions 1:300-1:500, which is suitable for most applications.

F(ab) fragments are quality control tested by UV-VIS spectroscopy and immunocytochemistry.



0.25mg
each F(ab)-Atto

ORDERING INFORMATION

Product	Kit Contents	Cat. #
Anti-Mouse IgG F(ab) -Atto488 Emission maximum: 523nm Immunofluorescence:1:300-500 Degree of labelling: 1-3	0.5mg (2mg/ml) anti-mouse F(ab) IgG (H+L) Atto488;* Product documentation; * in 50% glycerol, 0.01 M sodium phosphate, 0.1 M sodium chloride, 5 mM sodium azide, pH 7.4.	2112-250UG
Anti-Mouse IgG F(ab)-Atto565 Emission maximum: 592nm Immunofluorescence:1:300-500 Degree of labelling: 1-3	0.5mg (2mg/ml) anti-mouse F(ab) IgG (H+L) Atto565;* Product documentation; * in 50% glycerol, 0.01 M sodium phosphate, 0.1 M sodium chloride, 5 mM sodium azide, pH 7.4.	2117-250UG
Anti-Mouse IgG F(ab)-Atto594 Emission maximum: 627nm Immunofluorescence:1:300-500 Degree of labelling: 1-3	0.5mg (2mg/ml) anti-mouse F(ab) IgG (H+L) Atto594* Product documentation; * in 50% glycerol, 0.01 M sodium phosphate, 0.1 M sodium chloride, 5 mM sodium azide, pH 7.4.	2116-250UG
Anti-Mouse IgG F(ab)-Atto647N Emission maximum: 669nm Immunofluorescence:1:300-500 Degree of labelling: 1-3	0.5mg (2mg/ml) goat-anti mouse IgG (H+L) Atto647N;* Product documentation; * in 50% glycerol, 0.01 M sodium phosphate, 0.1 M sodium chloride, 5 mM sodium azide, pH 7.4.	2118-250UG
Anti-Mouse IgG F(ab)-Atto700 Emission maximum: 719nm Immunofluorescence:1:300-500 Degree of labelling: 1-3	0.5mg (2mg/ml) anti-mouse F(ab) IgG (H+L) Atto700* Product documentation; * in 50% glycerol, 0.01 M sodium phosphate, 0.1 M sodium chloride, 5 mM sodium azide, pH 7.4.	2110-250UG
Anti-Mouse IgG F(ab)-Atto740 Emission maximum: 764nm Immunofluorescence:1:300-500 Degree of labelling: 1-3	0.5mg (2mg/ml) anti-mouse F(ab) IgG (H+L) Atto740;* Product documentation; * in 50% glycerol, 0.01 M sodium phosphate, 0.1 M sodium chloride, 5 mM sodium azide, pH 7.4.	2111-250UG

Anti-Mouse IgM Atto produced in goat

Super Bright Detection of Monoclonal IgMs

The quality of secondary antibodies is essential in producing superior results in immunofluorescence detection. The affinity purified goat-anti mouse IgMs recognize with high specificity the H+L chain. Conjugated to Atto dyes - known for superior features compared to common dyes -, these antibodies are suited to serve highest demands in any type of fluorescence microscopy.

Fluorescent antibody kits are supplied with an epitope mapped monoclonal anti-actin for double staining or reference (1:100-1:300).

Affinity purified anti-mouse IgMs (1:300-1:1000) produced in goat, are quality control tested by UV-VIS spectroscopy and immunocytochemistry.

1.0mg
each IgM-Atto



ORDERING INFORMATION

Product	Kit Contents	Cat. #
Anti-Mouse IgM Atto390 Emission maximum: 479nm Immunofluorescence:1:300-1000 Degree of labelling: 2-9	1mg (2mg/ml) goat-anti mouse IgM (H+L) Atto390;* 50µg monoclonal anti-actin (epitope mapped);* Product documentation; Certificate of analysis; * in 50% glycerol, 0.01 M sodium phosphate, 0.1 M sodium chloride, 10 mM sodium azide, pH 7.4.	2201-1MG
Anti-Mouse IgM Atto488 Emission maximum: 523nm Immunofluorescence:1:300-1000 Degree of labelling: 2-9	1mg (2mg/ml) goat-anti mouse IgM (H+L) Atto488* 50µg monoclonal anti-actin (epitope mapped);* Product documentation; Certificate of analysis; * in 50% glycerol, 0.01 M sodium phosphate, 0.1 M sodium chloride, 10 mM sodium azide, pH 7.4.	2202-1MG
Anti-Mouse IgM Atto532 Emission maximum: 553nm Immunofluorescence:1:300-1000 Degree of labelling: 2-9	1mg (2mg/ml) goat-anti mouse IgM (H+L) Atto532;* 50µg monoclonal anti-actin (epitope mapped);* Product documentation; Certificate of analysis; * in 50% glycerol, 0.01 M sodium phosphate, 0.1 M sodium chloride, 10 mM sodium azide, pH 7.4.	2204-1MG
Anti-Mouse IgM Atto550 Emission maximum: 576nm Immunofluorescence:1:300-1000 Degree of labelling: 2-9	1mg (2mg/ml) goat-anti mouse IgM (H+L) Atto550;* 50µg monoclonal anti-actin (epitope mapped);* Product documentation; Certificate of analysis; * in 50% glycerol, 0.01 M sodium phosphate, 0.1 M sodium chloride, 10 mM sodium azide, pH 7.4.	2205-1MG



Anti-Mouse IgM Atto produced in goat

Super Bright Detection of Monoclonal IgMs

ORDERING INFORMATION

Product	Kit Contents	Cat. #
Anti-Mouse IgM Atto565 Emmission maximum: 592nm Immunofluorescence:1:300-1000 Degree of labelling: 2-9	1mg (2mg/ml) goat-anti mouse IgG (H+L) Atto565;* 50µg monoclonal anti-actin (epitope mapped);* Product documentation; Certificate of analysis; * in 50% glycerol, 0.01 M sodium phosphate, 0.1 M sodium chloride, 10 mM sodium azide, pH 7.4.	2207-1MG
Anti-Mouse IgM Atto594 Emmission maximum: 627nm Immunofluorescence:1:300-1000 Degree of labelling: 2-9	1mg (2mg/ml) goat-anti mouse IgM (H+L) Atto594;* 50µg monoclonal anti-actin (epitope mapped);* Product documentation; Certificate of analysis; * in 50% glycerol, 0.01 M sodium phosphate, 0.1 M sodium chloride, 10 mM sodium azide, pH 7.4.	2206-1MG
Anti-Mouse IgM Atto647 Emmission maximum: 669nm Immunofluorescence:1:300-1000 Degree of labelling: 2-9	1mg (2mg/ml) goat-anti mouse IgM (H+L) Atto647;* 50µg monoclonal anti-actin (epitope mapped);* Product documentation; Certificate of analysis; * in 50% glycerol, 0.01 M sodium phosphate, 0.1 M sodium chloride, 10 mM sodium azide, pH 7.4.	2208-1MG
Anti-Mouse IgM Atto700 Emmission maximum: 719nm Immunofluorescence:1:300-1000 Degree of labelling: 2-9	1mg (2mg/ml) goat-anti mouse IgM (H+L) Atto700;* 50µg monoclonal anti-actin (epitope mapped);* Product documentation; Certificate of analysis; * in 50% glycerol, 0.01 M sodium phosphate, 0.1 M sodium chloride, 10 mM sodium azide, pH 7.4.	2210-1MG
Anti-Mouse IgM Atto740 Emmission maximum: 764nm Immunofluorescence:1:300-1000 Degree of labelling: 2-9	1mg (2mg/ml) goat-anti mouse IgM (H+L) Atto740;* 50µg monoclonal anti-actin (epitope mapped);* Product documentation; Certificate of analysis; * in 50% glycerol, 0.01 M sodium phosphate, 0.1 M sodium chloride, 10 mM sodium azide, pH 7.4.	2211-1MG

Anti-Mouse IgM Atto produced in goat

Anti-Rabbit IgG Atto produced in goat

High Sensitive Immunofluorescence Detection of Polyclonal Primary Antibodies

The quality of secondary antibodies is essential in producing superior results in immunofluorescence detection. To obtain best results with anti-rabbit IgGs, we recommend the use of monospecific polyclonal antibodies to minimize unspecific side reactions. The affinity purified goat-anti mouse IgGs recognize with high specificity the H+L chain. Conjugated to Atto dyes with superior features as compared to common dyes, these antibodies are suited to serve highest demands in fluorescence microscopy.

Fluorescent antibody kits are supplied with an epitope mapped monoclonal anti-actin for double staining or re-

Affinity purified goat-anti rabbit IgGs (1:300-1:1000) are quality control tested by UV-VIS spectroscopy and immunocytochemistry.



1.0mg
each IgG-Atto



ORDERING INFORMATION

Product	Kit Contents	Cat. #
Anti-Rabbit IgG Atto390 Emission maximum: 479nm Immunofluorescence:1:300-1000 Degree of labelling: 2-9	1mg (2mg/ml) goat-anti rabbit IgG (H+L) Atto390;* 50µg monoclonal anti-actin (epitope mapped);* Product documentation; Certificate of analysis; * in 50% glycerol, 0.01 M sodium phosphate, 0.1 M sodium chloride, 10 mM sodium azide, pH 7.4.	2301-1MG
Anti-Rabbit IgG Atto488 Emission maximum: 523nm Immunofluorescence:1:300-1000 Degree of labelling: 2-9	1mg (2mg/ml) goat-anti rabbit IgG (H+L) Atto488* 50µg monoclonal anti-actin (epitope mapped);* Product documentation; Certificate of analysis; * in 50% glycerol, 0.01 M sodium phosphate, 0.1 M sodium chloride, 10 mM sodium azide, pH 7.4.	2302-1MG
Anti-Rabbit IgG Atto532 Emission maximum: 553nm Immunofluorescence:1:300-1000 Degree of labelling: 2-9	1mg (2mg/ml) goat-anti rabbit IgG (H+L) Atto532;* 50µg monoclonal anti-actin (epitope mapped);* Product documentation; Certificate of analysis; * in 50% glycerol, 0.01 M sodium phosphate, 0.1 M sodium chloride, 10 mM sodium azide, pH 7.4.	2304-1MG
Anti-Rabbit IgG Atto550 Emission maximum: 576nm Immunofluorescence:1:300-1000 Degree of labelling: 2-9	1mg (2mg/ml) goat-anti rabbit IgG (H+L) Atto550;* 50µg monoclonal anti-actin (epitope mapped);* Product documentation; Certificate of analysis; * in 50% glycerol, 0.01 M sodium phosphate, 0.1 M sodium chloride, 10 mM sodium azide, pH 7.4.	2305-1MG

Anti-Rabbit IgG Atto produced in goat

High Sensitive Immunofluorescence Detection
of Polyclonal Primary Antibodies



ORDERING INFORMATION

Product	Kit Contents	Cat. #
Anti-Rabbit IgG Atto565 Emission maximum: 669nm Immunofluorescence:1:300-1000 Degree of labelling: 2-9	1mg (2mg/ml) goat-anti rabbit IgG (H+L) Atto565;* 50µg monoclonal anti-actin (epitope mapped);* Product documentation; Certificate of analysis; * in 50% glycerol, 0.01 M sodium phosphate, 0.1 M sodium chloride, 10 mM sodium azide, pH 7.4.	2307-1MG
Anti-Rabbit IgG Atto594 Emission maximum: 627nm Immunofluorescence:1:300-1000 Degree of labelling: 2-9	1mg (2mg/ml) goat-anti rabbit IgG (H+L) Atto594;* 50µg monoclonal anti-actin (epitope mapped);* Product documentation; Certificate of analysis; * in 50% glycerol, 0.01 M sodium phosphate, 0.1 M sodium chloride, 10 mM sodium azide, pH 7.4.	2106-1MG
Anti-Rabbit IgG Atto647N Emission maximum: 669nm Immunofluorescence:1:300-1000 Degree of labelling: 2-9	1mg (2mg/ml) goat-anti rabbit IgG (H+L) Atto647;* 50µg monoclonal anti-actin (epitope mapped);* Product documentation; Certificate of analysis; * in 50% glycerol, 0.01 M sodium phosphate, 0.1 M sodium chloride, 10 mM sodium azide, pH 7.4.	2308-1MG
Anti-Rabbit IgG Atto700 Emission maximum: 719nm Immunofluorescence:1:300-1000 Degree of labelling: 2-9	1mg (2mg/ml) goat-anti rabbit IgG (H+L) Atto700;* 50µg monoclonal anti-actin (epitope mapped);* Product documentation; Certificate of analysis; * in 50% glycerol, 0.01 M sodium phosphate, 0.1 M sodium chloride, 10 mM sodium azide, pH 7.4.	2310-1MG
Anti-Rabbit IgG Atto740 Emission maximum: 764nm Immunofluorescence:1:300-1000 Degree of labelling: 2-9	1mg (2mg/ml) goat-anti rabbit IgG (H+L) Atto740;* 50µg monoclonal anti-actin (epitope mapped);* Product documentation; Certificate of analysis; * in 50% glycerol, 0.01 M sodium phosphate, 0.1 M sodium chloride, 10 mM sodium azide, pH 7.4.	2311-1MG

Anti-Rabbit IgG Atto produced in goat

Anti-Rabbit IgG F(ab) Atto produced in goat

For Double Staining or Inaccessible Structures

F(ab) fragments are generated by limited cleavage with papain from affinity purified goat-anti rabbit IgGs, that recognize with high specificity the H+L chain. Conjugated to Atto dyes - known for superior features compared to common dyes -, F(ab) fragments are suited to serve highest demands using any type of fluorescence microscopy.

Anti-Rabbit IgG (H+L) F(ab) are used in dilutions 1:300-1:500, which is suitable for most applications.

F(ab) fragments are quality control tested by UV-VIS spectroscopy and immunocytochemistry.

0.25mg
each F(ab)-Atto



ORDERING INFORMATION

Product	Kit Contents	Cat. #
Anti-Rabbit IgG F(ab) -Atto488 Emission maximum: 523nm Immunofluorescence:1:300-500 Degree of labelling: 1-3	0.5mg (2mg/ml) anti-Rabbit F(ab) IgG (H+L) Atto488;* Product documentation; * in 50% glycerol, 0.01 M sodium phosphate, 0.1 M sodium chloride, 5 mM sodium azide, pH 7.4.	2312-250UG
Anti-Rabbit IgG F(ab)-Atto565 Emission maximum: 592nm Immunofluorescence:1:300-500 Degree of labelling: 1-3	0.5mg (2mg/ml) anti-Rabbit F(ab) IgG (H+L) Atto565;* Product documentation; * in 50% glycerol, 0.01 M sodium phosphate, 0.1 M sodium chloride, 5 mM sodium azide, pH 7.4.	2317-250UG
Anti-Rabbit IgG F(ab)-Atto594 Emission maximum: 627nm Immunofluorescence:1:300-500 Degree of labelling: 1-3	0.5mg (2mg/ml) anti-Rabbit F(ab) IgG (H+L) Atto594* Product documentation; * in 50% glycerol, 0.01 M sodium phosphate, 0.1 M sodium chloride, 5 mM sodium azide, pH 7.4.	2316-250UG
Anti-Rabbit IgG F(ab)-Atto647N Emission maximum: 669nm Immunofluorescence:1:300-500 Degree of labelling: 1-3	0.5mg (2mg/ml) goat-anti Rabbit IgG (H+L) Atto647N;* Product documentation; * in 50% glycerol, 0.01 M sodium phosphate, 0.1 M sodium chloride, 5 mM sodium azide, pH 7.4.	2318-250UG
Anti-Rabbit IgG F(ab)-Atto700 Emission maximum: 719nm Immunofluorescence:1:300-500 Degree of labelling: 1-3	0.5mg (2mg/ml) anti-Rabbit F(ab) IgG (H+L) Atto700* Product documentation; * in 50% glycerol, 0.01 M sodium phosphate, 0.1 M sodium chloride, 5 mM sodium azide, pH 7.4.	2310-250UG
Anti-Rabbit IgG F(ab)-Atto740 Emission maximum: 764nm Immunofluorescence:1:300-500 Degree of labelling: 1-3	0.5mg (2mg/ml) anti-Rabbit F(ab) IgG (H+L) Atto740;* Product documentation; * in 50% glycerol, 0.01 M sodium phosphate, 0.1 M sodium chloride, 5 mM sodium azide, pH 7.4.	2311-250UG

FluoDuo

Your selection - your choice!
Customized Atto secondary antibody kits.

Choose between two affinity purified Atto-conjugated secondary antibodies for double immunostaining, FRET or the application of your choice. A selection of our most popular antibodies is now available in smaller quantities.

The affinity purified IgGs recognize with high specificity the H+L chain. Conjugated to Atto dyes known for superior features compared to common dyes, the antibodies are suited to serve highest demands in any type of fluorescence microscopy.

Convenient double staining with next generation Atto-fluorescent antibodies.



Antibody 1

Affinity purified anti-mouse IgGs (1:500-1:1500) produced in goat are quality control tested by UV-VIS spectroscopy and immunocytochemistry.

Antibody 2

Affinity purified anti rabbit IgGs (1:300-1:1000) produced in goat are quality control tested by UV-VIS spectroscopy and immunocytochemistry.

ORDERING INFORMATION

Antibody 1		Antibody 2	Cat. #
gam Atto390	Mix your kit with 2 antibodies from antibody 1 & 2 as you like.	gar Atto390	2700-00
gam Atto488		gar Atto488	2700-00
gam Atto514		gar Atto514	2700-00
gam Atto532		gar Atto532	2700-00
gam Atto565		gar Atto565	2700-00
gam Atto594		gar Atto594	2700-00
gam Atto647		gar Atto647	2700-00

FluoDuo kits all have the same Cat. #!
Please state in your order which combination you like.

Determination of the Degree of Labeling (DOL) for Atto-fluorescent IgGs

The Extinction Coefficient (in Mol⁻¹cm⁻¹)

links the quantity of absorbed light, at a given wavelength, to the concentration of fluorophore in solution.

The degree of labeling (DOL or dye/protein ratio) is frequently determined by absorption spectroscopy according to Lambert-Beer: Absorbance (A) = extinction coefficient (ε) × molar concentration × path length (d). The UV-VIS spectrum of the conjugate in solution is measured in a quartz cuvette. Dilute the solution, if necessary to measure within the linear range according to the Lambert-Beer law. Determine the absorbance (A_{max}) at the absorption maximum (λ_{abs}) of the dye and the absorbance (A₂₈₀) at 280 nm (absorption maximum of proteins). The concentration of bound dye is given by:

$c(\text{dye}) = A_{\text{max}} / \epsilon_{\text{max}} \times d$,
 ϵ_{max} = extinction coefficient of the dye at the absorption maximum.

The protein concentration is determined at 280 nm. All dyes show some absorption at 280nm. The absorbance measured at A₂₈₀ thus must be corrected for the contribution of the dye.

This is given by $A_{\text{max}} \times \text{CF}_{280}$ (CF₂₈₀ = correction factor at 280 nm) = $\epsilon_{280} / \epsilon_{\text{max}}$

It follows for the absorbance of the protein itself:

$A_{\text{prot}} = A_{280} - A_{\text{max}} \times \text{CF}_{280}$.

The protein concentration is: $c(\text{protein}) = A_{\text{prot}} / \epsilon_{\text{prot}} \times d$ (ϵ_{prot} = extinction coefficient of the protein at 280 nm).

From this it follows for the degree of labeling, i.e. the average number of dye molecules covalently bound to an IgG: $\text{DOL} = c(\text{dye}) / c(\text{protein})$ using the above relations:

$$\text{DOL} = \frac{A_{\text{max}} / \epsilon_{\text{max}}}{A_{\text{prot}} / \epsilon_{\text{prot}}} = \frac{A_{\text{max}} \cdot \epsilon_{\text{prot}}}{A_{280} - (A_{\text{max}} \cdot \text{CF}_{280}) \cdot \epsilon_{\text{max}}}$$

Optical Properties of Atto dyes

Label	λ _{abs} (nm)	λ _{fl} (nm)	Substitutes for	Examples of suitable light sources
Atto390	390	479		Mercury arc lamp
Atto488	501	523	Alexa488 ¹⁾ , FITC, FAM ²⁾	Argon-Ion laser
Atto514	511	533	Alexa514 ¹⁾	Argon-Ion laser
Atto532	532	553	Alexa532 ¹⁾ , HEX ²⁾	Argon-Ion laser, Nd:YAG laser
Atto550	554	576	TAMRA ²⁾ , Cy3 ³⁾	Mercury arc lamp, Argon-Ion laser, Nd:YAG laser
Atto565	563	592	Cy3.5 ³⁾ , ROX	Nd:YAG laser,
Atto594	601	627	Alexa594 ¹⁾	Mercury arc lamp
Atto633	629	657	Cy5 ³⁾ , Alexa633 ¹⁾	He-Ne laser, Diode laser
Atto647	645	669	Cy5 ³⁾ , Alexa647 ¹⁾	He-Ne laser, Diode laser, Krypton-Ion laser
Atto647N	644	669	Cy5 ³⁾ , Alexa647 ¹⁾	He-Ne laser, Diode laser, Krypton-Ion laser
Atto680	680	700	Cy5.5 ³⁾	Krypton-Ion laser
Atto700	700	719	Cy5.5 ³⁾	Krypton-Ion laser
Atto740	740	764		Krypton-Ion laser

¹⁾ Trademark of Invitrogen Corporation, ²⁾ Trademark of Applera Corporation, ³⁾ Trademark of GE Healthcare Group Companies

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Antibodies are shipped at ambient temperature and contain a glycerol buffer for reconstitution upon arrival. They are stable for at least 1 year, when frozen as a glycerol stock at -20°C . At best prepare small aliquots prior to freezing and use each aliquot without re-freezing.

Within Germany all antibodies are shipped as liquid. To guarantee highest quality, orders estimated to be shipped $>24\text{h}$, are supplied as freeze-dried products, containing a glycerol solution for convenient reconstitution upon arrival. Freeze-dried antibodies are shipped at ambient temperature (shipping rates see below).

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Atto647N

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