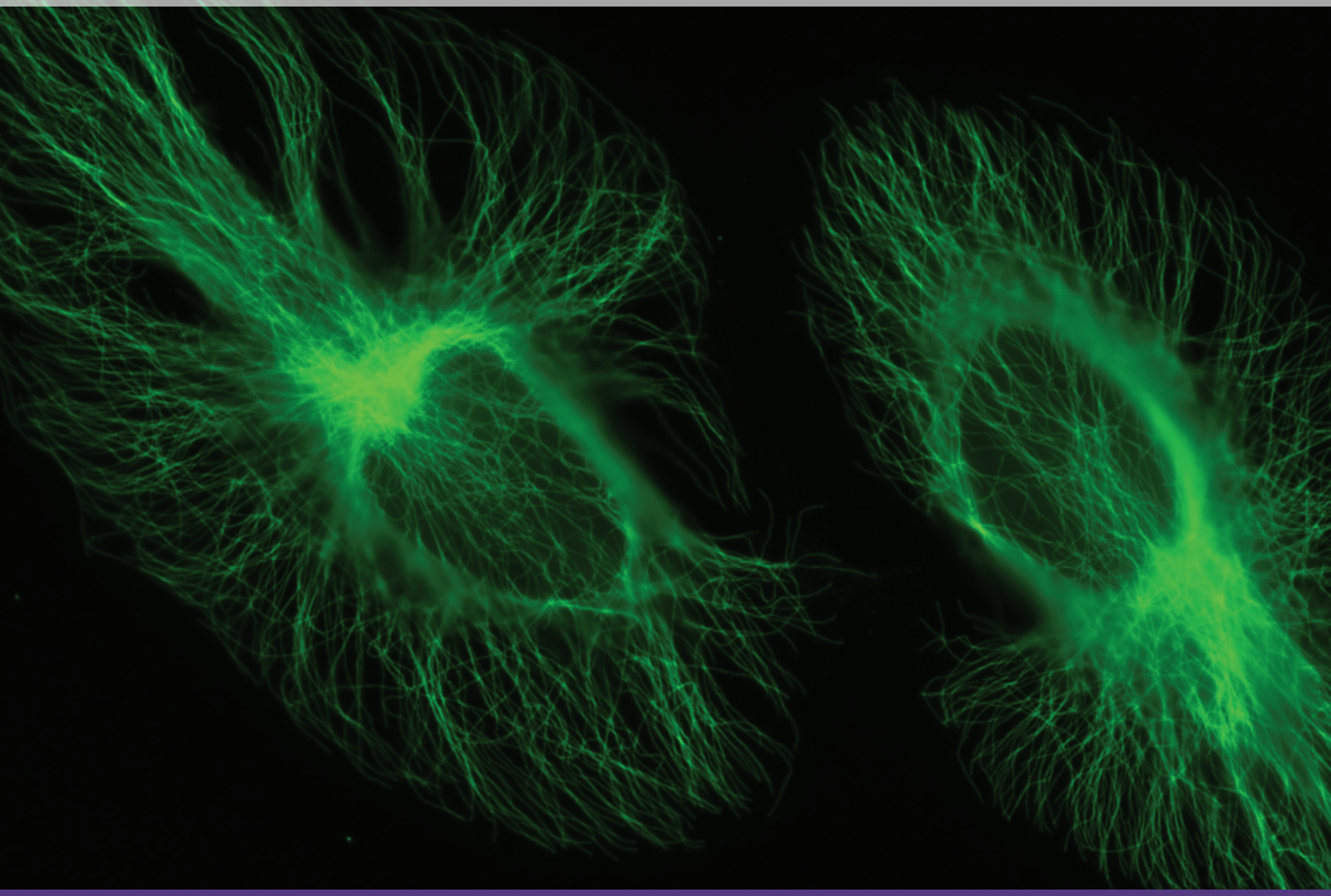

Chromeo™

complete tool set for successful fluorescence applications

Fluorescent Chromeo™ Dyes and Labeling Kits

Fluorescent Secondary Antibody Conjugates

Reagents for STED Super-resolution Microscopy

Chromeo™ Py-Dyes – Fluorescent Chameleons

Fluorescent Assays

Active Motif's Chromeo™ product line provides researchers with high-quality fluorescent dyes that have superior fluorescent properties, optimized reagents that have been tested and verified to work in various applications, as well as a number of innovative cell biology assays.

Use Chromeo products to simplify the preparation of high-quality images in fluorescence microscopy, or to help you develop your own fluorescent assays.

ACTIVE MOTIF®

Tools to Analyze Nuclear Function

Chromeo™ Fluorescent Dyes

Active Motif's Chromeo™ Dyes – Chromeo™ 488, Chromeo™ 494, Chromeo™ 505, Chromeo™ 546 and Chromeo™ 642 – are used to detect biomolecules in a variety of assays. They are typically used to label biomolecules covalently in a chemical reaction. To give you more choices when labeling, Chromeo Dyes are offered in 6 different formats: as amine-reactive NHS-esters, which are useful to label amino groups on peptides, proteins or amino-modified DNA; as azides and alkynes for use in click chemistry; as carboxylic acids, and as biotin and streptavidin conjugates. For application data and ordering information for the various dye formats, please visit us at www.activemotif.com/dyes.

For the fluorescent properties of each dye, please see Table 1 on the next page.

For the utmost in convenience, the dyes are available already conjugated to high-quality goat anti-mouse and goat anti-rabbit secondary antibodies (see next page).

Why use Chromeo™ Dyes?

- **Brightness** – high fluorescent intensity improves sensitivity
- **Limited photobleaching** – enables multiple exposures and increased exposure times
- **pH stability** – great for biological assays
- **Instrument compatibility** – work with common excitation sources like diode lasers, LEDs, tungsten and xenon lamps
- **Flexible formats** – available as NHS-Esters, Azides, Alkynes, Carboxylic Acids, and as Biotin, Streptavidin & secondary antibody conjugates
- **Easy to use** – room temperature incubations & no harsh chemicals required for conjugation

Fluorescent Labeling Kits

Active Motif's Chromeo™ Antibody Labeling Kits make it simple to label your antibodies and proteins with the Chromeo Dye of your choice. Each kit provides lyophilized Chromeo 488, 494, 546 or 642 Dye in three convenient, one-time-use vials that will label 1 mg of antibody/protein each. Also included are purification columns and the buffers required for each labeling reaction.

Product	Format	Catalog No.
Chromeo™ 488 Antibody Labeling Kit	1 kit	15090
Chromeo™ 494 Antibody Labeling Kit	1 kit	15091
Chromeo™ 546 Antibody Labeling Kit	1 kit	15092
Chromeo™ 642 Antibody Labeling Kit	1 kit	15093

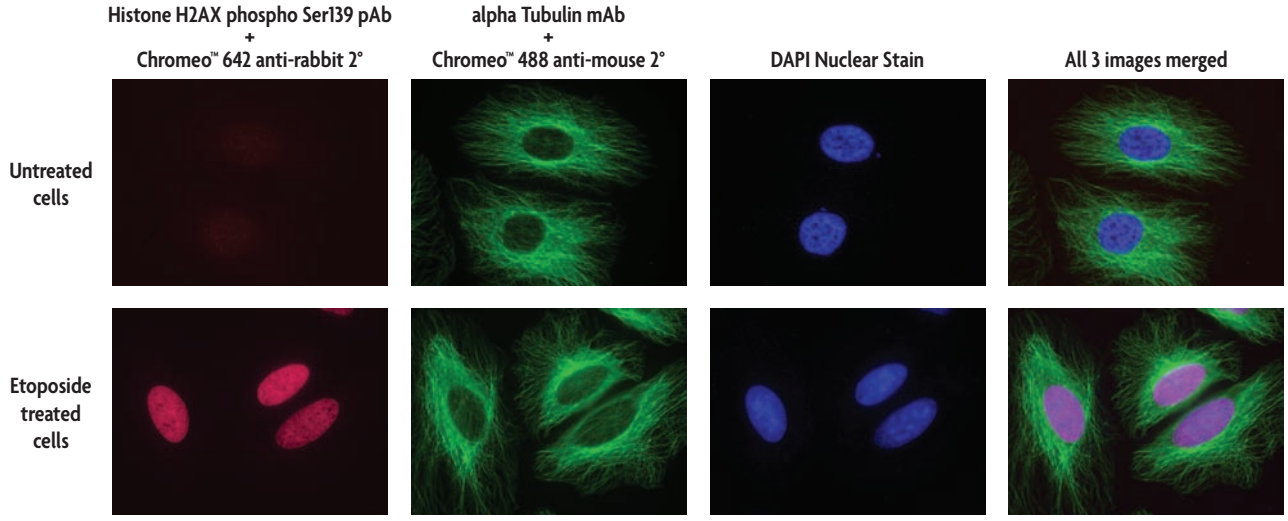

Figure 1: Concurrent staining using Chromeo 488 and Chromeo 642 fluorescent secondaries in untreated and etoposide-treated HeLa cells.
HeLa cells were either left untreated, or treated with 100 µM etoposide for 6 hours, prior to fixation with methanol. The histone variant H2AX was then stained with Histone H2AX phospho Ser139 rabbit pAb (Cat. No. 39117) and Chromeo 642 goat anti-rabbit IgG (Cat. No. 15044), while tubulin was visualized using alpha Tubulin mouse mAb (Cat. No. 39527) and Chromeo 488 goat anti-mouse IgG (Cat. No. 15031). The nuclei were stained using DAPI and the three separate images were merged.

Fluorescent secondary antibodies

To help ensure you get the best quality fluorescence microscopy images possible, Active Motif offers high-quality goat anti-mouse and goat anti-rabbit secondary antibodies that have been conjugated to a wide variety of fluorescent dyes, including Chromeo™ Dyes. In addition, we offer ATTO conjugates that have been maximally cross-adsorbed against the IgG's of a variety of species; this helps to eliminate background caused by non-specific binding. Because of the wide range of dyes offered (see Table 1), you can use multiple secondaries at the same time for multi-color staining (see Figure 1, previous page). HRP conjugates are also available.

Why use Active Motif secondaries?

For fluorescent detection of primary antibodies, it is critical that the secondary antibody bind whether the cells are treated with methanol, formaldehyde or even formalin. Active Motif's fluorescent secondaries have been tested and shown to work with high efficiency and specificity under all of the most commonly used fixation conditions.

In addition to the spectral properties of the dye and the quality of the secondary antibody, the quality of a fluorescent conjugate is influenced by the dye-to protein ratio, the conjugation method and its purity. Active Motif's optimized conjugation method, coupled with subsequent purification of the conjugate from interfering unbound dye molecules, makes our fluorescent secondaries brighter than other commercially available conjugates and lowers the background in many applications. To ensure your success, Active Motif antibody conjugates have been tested in various applications including flow cytometry and widefield or confocal fluorescence microscopy.

Dye	Absorption	Emission	ε L/(mol-cm)	Stokes Shift
Chromeo™ 488	498 nm	524 nm	73,000	26 nm
Chromeo™ 494	489 nm	624 nm	55,000	135 nm
Chromeo™ 505	514 nm	530 nm	70,000	16 nm
Chromeo™ 546	550 nm	567 nm	98,800	17 nm
Chromeo™ 642	647 nm	666 nm	180,000	19 nm
ATTO 594	601 nm	627 nm	120,000	26 nm
ATTO 647N (STED)	644 nm	669 nm	150,000	25 nm
ATTO 655 (STED)	663 nm	684 nm	125,000	21 nm

Table 1: Fluorescent properties of dyes when conjugated to secondary antibodies.
All dyes are offered conjugated to both Goat anti-mouse IgG and Goat anti-rabbit IgG secondary antibodies in regular and sample sizes. HRP conjugates are also available. Please visit www.activemotif.com/secondary for details and ordering information.

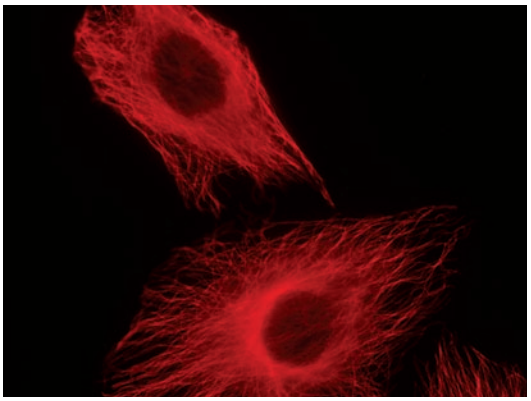

Figure 2: Chromeo 494 staining in HeLa cells.
HeLa cells were stained with rabbit alpha Tubulin polyclonal antibody and Chromeo 494 Goat anti-rabbit IgG (Cat. No. 15042) using MAX Stain reagents.

MAX Stain™ Immunofluorescence Tools

MAX Stain™ Immunofluorescence Tools eliminate the challenge of getting high-quality fluorescent images consistently by providing a complete set of optimized reagents. MAX Stain reagents are used in our daily work to improve the quality of our experiments (Figure 2).

- **MAXblock™ Blocking Medium** is a non-mammalian, protein-based blocking agent for use in immunofluorescence and immunohistochemistry. It effectively blocks non-specific antibody binding without reducing signal intensity or specificity.
- **MAXwash™ Washing Medium** includes a non-mammalian incubation agent that promotes superior antibody binding while eliminating non-specific primary and secondary antibody binding.
- **MAXbind™ Staining Medium** is a proprietary formulation designed and optimized to increase antibody binding specificity and intensity of your IF experiments.

Product	Format	Catalog No.
MAXpack™ Immunostaining Media Kit (includes one each of 15252, 15253, & 15254)	1 kit	15251
MAXblock™ Blocking Medium	150 ml	15252
MAXbind™ Staining Medium	250 ml	15253
MAXwash™ Washing Medium	1000 ml	15254

Get 12-fold greater resolution with STimulated Emission Depletion (STED) microscopy

The *Abbe Law of Diffraction Limiting Resolution* restricts the ability of classical confocal microscopy to visually resolve objects separated by less than ~200 nm. Leica Microsystems' **ST**imulated **E**mission **D**epletion (**STED**) microscopy, however, improves resolution up to 12-fold, enabling separation of sub-cellular structures that previously could not be resolved. To help ensure your success in STED microscopy, Active Motif collaborated with Leica to create a kit that optimizes the preparation of samples for STED. In addition, Leica has tested and certified a number of Active Motif's fluorescent dyes. For more details on our STED products, please visit us at www.activemotif.com/sted.

Optimized sample preparation for STED microscopy

Proper sample preparation is perhaps the most significant factor for obtaining clear, conclusive, high-resolution images when performing STED microscopy. To help ensure that you consistently achieve the best results possible, Active Motif worked with Leica Microsystems to develop the **Chromeo™ STED Immunofluorescence System**. This kit helps take the guesswork and challenge out of preparing samples for STED microscopy by providing a complete set of proven, QC-tested reagents and an optimized protocol. Using the kit is an inexpensive way to be certain you obtain the most scientific value from the investment in your STED microscope, and the reason Leica recommends the kit for use with its STED microscopes.

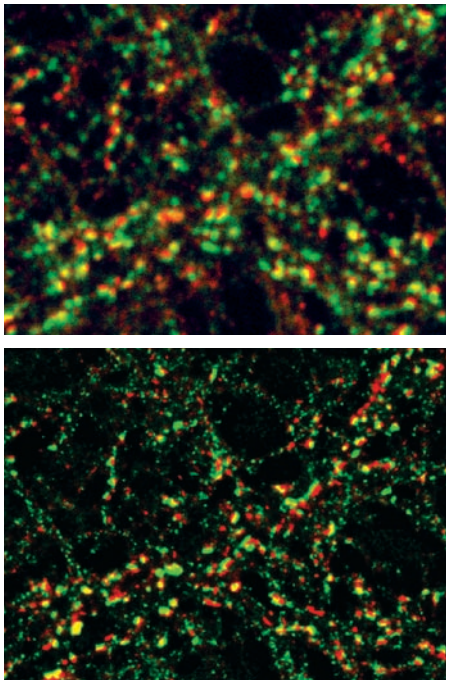

Figure 3: Confocal and STED microscopy of pre- and post-synaptic marker proteins.
The localization of pre- and post-synaptic marker proteins visualized in the dendrites of nerve cells using conventional, confocal (top image) and STED microscopy (bottom image). The pre-synaptic protein Bassoon was stained by ATTO 647N (red), while the post-synaptic protein Homer was stained with Chromeo 494 (green). Images courtesy of Dr. W. Zuschratter, IfN Magdeburg, Germany.

Fluorescent dyes recommended by Leica for use in STED

In collaborating to help create the Chromeo™ STED Immunofluorescence System, Leica tested and now recommends the following Active Motif fluorescent dyes and conjugates for STED:

- Chromeo 488 dyes and secondary antibody conjugates are recommended for use with the Leica **TCS STED CW** microscope, which contains a continuous argon gas laser for excitation.
- Active Motif's secondary conjugates of ATTO 647N and ATTO 655 (STED) have been certified by Leica for use with the 640 nm laser of the Leica **TCS STED** microscope.
- Chromeo 494 dye and secondary antibody conjugates meet the specifications of an **upgraded TCS STED** microscope that includes a second excitation laser of 531 nm, enabling dual-color, high-resolution microscopy (Figure 3). The unique fluorescent properties of Chromeo 494, such as its large Stokes shift (Figure 4), enable you to perform co-localization studies at resolutions below the diffraction limit. This new method makes it possible to view the nuclear architecture or structures at the synapses in more detail, which will help in the study and discovery of sub-cellular structures and processes.

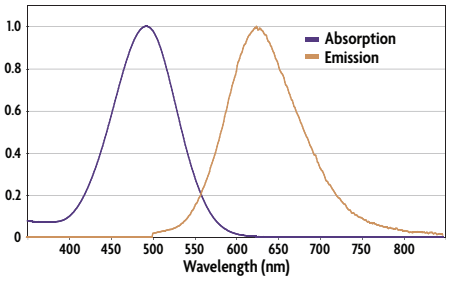

Figure 4: Absorption/Emission of Chromeo™ 494.

Effective fluorescent labels for Click chemistry

Click chemistry constitutes a novel approach for easy, effective labeling and detection of biomolecules *in vitro* and *in vivo*. It is based on the use of biologically unique tags as reaction partners. The use of **azide** and **alkyne** tags for labeling proteins, nucleic acids, lipids or sugars has the advantage that neither moiety occurs in nature, and neither reacts with other molecules in cellular systems. Because of its high selectivity, bio-orthogonal click chemistry can be used for detection in complex biological samples: one of the reaction partners is integrated into the molecule of interest, while the second tag containing a fluorescent label is covalently attached by performing the click reaction. Chromeo 488, Chromeo 494, Chromeo 546 and Chromeo 642 Dyes are offered as click-reactive azides and alkynes for use in this highly specific labeling technology.

Chromeo™ Py-Dyes – simple-to-use chameleon dyes for effective protein labeling

Chromeo™ Py-Dyes are a unique class of amine-reactive, fluorescent labels with a number of properties that make them ideal for labeling proteins. Unlike most other labels, Py-Dyes undergo significant changes in their spectral properties when conjugated to protein. Upon binding to primary amine groups, Py-Dyes exhibit a shortwave spectral shift as well as a large increase in quantum yield; consequently, only the conjugates emit detectable fluorescence (Table 2 & Figure 5). In addition, because unbound Py-Dye is hydrolyzed during the labeling procedure, there is no need for purification following the simple one-step, room-temperature incubation. Labeled protein is ready to use immediately, and any possible background from unbound dye is eliminated. Finally, the positive charge of the amine group is maintained after Py-Dye conjugation, so labeled protein maintains the same ionic character as the native protein (Figure 6).

Py-Dye applications

Because of their unique properties and advantages, Py-Dyes can be used in many different applications:

- Pre- and Post-stain in protein gel-electrophoresis
- Monitoring of amino bonds on surfaces
- Stain in capillary electrophoresis (CE)
- Receptor binding studies
- Protein Quantification
- Stain in capillary iso-electric focusing (CIEF)

Why label with Py-Dyes?

- Broad Stokes shifts and the automatic degradation of unbound dye during conjugation eliminate background
- Large increase in quantum yield upon binding causes the non-fluorescent dye to become fluorescent
- No change to the net charge of the protein after labeling
- Dye is supplied ready to label in a fast, simple procedure with no wash steps, or need to purify the conjugated protein

Figure 5: Spectral shift of Py-Dyes following conjugation to protein.
The emission of Chromeo P503 in its unbound, free state is blue. Following conjugation to protein, the color of the dye changes to red and any unbound dye automatically degrades.

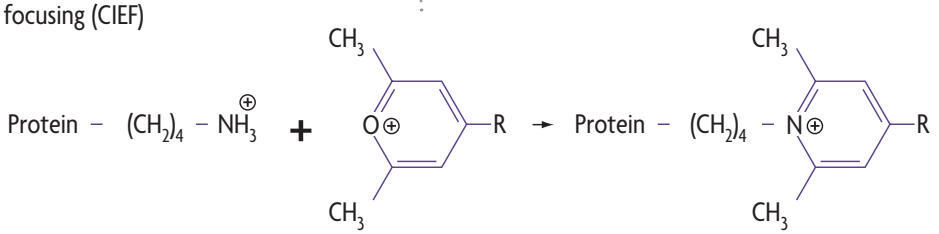

Figure 6: Labeling with Py-Dyes maintains each protein's natural charge.
Chemical equation depicting the chemistry of Py-Dye labeling, which demonstrates the preservation of the positive charge of the lysine residue of a protein. R is the respective chromogenic/fluorogenic group.

Dye	Dye State	Absorption	Emission	ε L/ (mol-cm)	Quantum Yield*
Chromeo™ P465	Unbound	645 nm	732 nm	25,000	< 1%
	Conjugated	465 nm	630 nm	25,000	~14%
Chromeo™ P503	Unbound	612 nm	665 nm	60,000	< 1%
	Conjugated	503 nm	600 nm	24,000	~50%
Chromeo™ P540	Unbound	587 nm	–	80,000	0%
	Conjugated	533 nm	627 nm	50,000	~20%
Chromeo™ P429	Unbound	457 nm	–	65,000	0%
	Conjugated	429 nm	536 nm	75,000	~10%
Chromeo™ P543	Unbound	570 nm	–	110,000	0%
	Conjugated	543 nm	590 nm	57,000	~15%

Product	Format	Catalog No.
Chromeo™ P465	1 mg	15105
	5 mg	16105
Chromeo™ P503	1 mg	15106
	5 mg	16106
Chromeo™ P540	1 mg	15107
	5 mg	16107
Chromeo™ P429	1 mg	15108
	5 mg	16108
Chromeo™ P543	1 mg	15109
	5 mg	16109

Table 2: Properties of Chromeo Py-Dyes.
*Quantum Yield will depend on the Dye-to-Protein Ratio (DPR) of the conjugated protein.

Albumin Blue Fluorescent Assay Kit

The Albumin Blue Fluorescent Assay Kit is a quantitative assay for measuring albumin levels in biological samples, such as serum and urine. The kit combines unmatched sensitivity, high specificity and a fast protocol to provide an effective way to study albumin.

Other assays for determining low albumin concentrations (< 100 mg/L) are non-specific, and merely quantify total protein. In contrast, Active Motif's Albumin Assay Kit is sensitive and specific for albumin. Simply add the Diluent Buffer and Dye Reagent to the standards and samples, then read the fluorescence. The intensity of the fluorescent signal is directly proportional to the albumin concentration of the sample. And, as albumin-bound dye has a greatly increased excitation, the background caused by the emission of any free dye is minimal (Figure 7).

Each kit contains dye for 250 reactions in 96-well plates; it can also be used in smaller or larger formats, such as 384-well plates or cuvettes. The kit also includes human serum albumin (HSA) to make standard curves for quantifying the albumin in your samples (Figure 8). As albumin is highly conserved, the kit can detect albumin from various species.

Why use Albumin Blue?

- Quick and easy to use
- Unmatched precision
- Sensitive detection limit (< 0.5 mg/L in urine and serum samples)
- Highly selective – no interference from other proteins or lipids
- High-throughput compatible

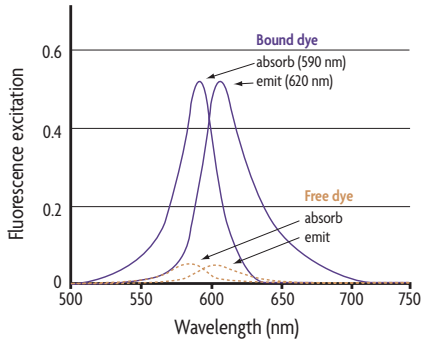

Figure 7: Absorption and emission spectra of dye that is free or bound to albumin.
Normalized absorption and emission spectra of free (copper curves) and conjugated dye (purple curves) in the Albumin Blue Assay.

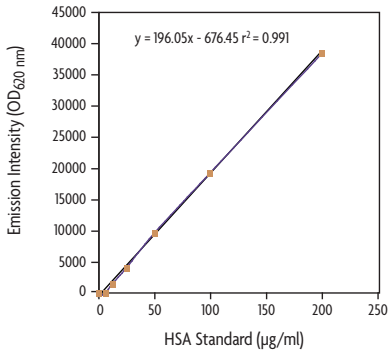

Figure 8: Human Serum Albumin standard curve.
An HSA standard curve generated using the control included in the Albumin Blue Fluorescent Assay Kit.

Product	Format	Catalog No.
Albumin Blue Fluorescent Assay Kit	1 kit	15002

Cell and Organelle Stains

Active Motif offers several products to study cellular structure using fluorescence microscopy or high-content applications. Our Cell and Organelle Stains are a collection of innovative fluorescent dyes that provide specific and efficient staining of subcellular structures in live and in fixed cells. A key feature is that the dyes become fluorescent only after specific binding to target structures; this eliminates background fluorescence caused by any unbound dye.

- **LavaCell™** is a naturally fluorescent compound that diffuses into live or fixed cells, where it stains the plasma membrane and internal membranes, such as those of the nucleus, Golgi and ER.
- **Chromeo™ Live Cell Mitochondrial Staining Kit** is a membrane permeable dye that selectively stains mitochondria in living cells. The low toxicity and the retention within the cell and the photostability makes this dye an ideal tool for long-term labeling of cells and cellular tracking.
- **Chromeo™ Red Fluorescent Fixed Cell Staining Kit** serves as a counterstain in immunofluorescence experiments where its red color can be used in combination with green dyes such as Chromeo™ 488, FITC or other 488-excitable dyes.

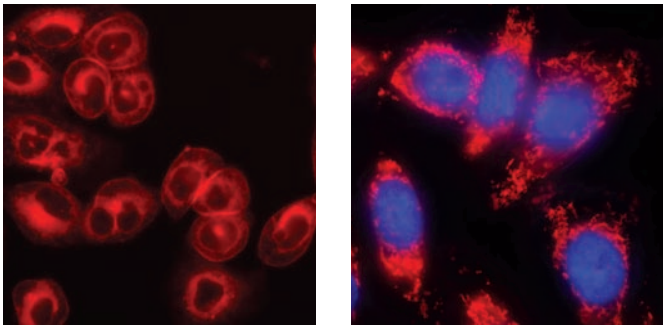

Figure 9: Live cell staining with Active Motif Cell and Organelle Stains.
On the left: LavaCell stains the plasma membrane and internal membranes of CHO cells. On the right: Staining of mitochondria (red) and the nuclei (blue) in living HeLa cells with the Mitochondrial Dye and Hoechst nuclear stain that are included in the Chromeo Live Cell Mitochondrial Staining Kit.

Product	Format	Catalog No.
LavaCell™ Live Cell Membrane Staining Kit	200 µg	15004
Chromeo™ Live Cell Mitochondrial Staining Kit	1 kit	15005
Chromeo™ Red Fluorescent Fixed Cell Staining Kit	1 kit	15006