Keep your research on track and moving forward.

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Western Blotting Solutions

The highest sensitivity has arrived

With applications in biotechnology, molecular biology, proteomics, and more, western blotting is the most widely used technique for detecting protein expression levels in cells. Using a unique combination of specific immunodetection and size-based separation capabilities, western blotting provides reliable, convenient, high-quality data that's considered to be the gold standard in protein detection.

Our wide range of western blotting solutions gives you the highest level of sensitivity, specificity, and selectivity so you can be confident in your results.

From products that enhance western blotting performance to our deep-seated knowledge and expertise, we offer the tools you need to find the answers you want.

Keep your analysis running on time, every time.

Stay on track with our Western Blotting Solutions

- Offers the ultimate in sensitivity
- Delivers robust detection under a variety of conditions
- Offers reduced consumption of antibodies and samples
- Features a wide dynamic range
- Supplies a range of detection methods

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Stay the course with a proven Western Blotting workflow

Researchers have depended on western blotting for more than 40 years. With continued advancements in materials and technology, combined with our newest portfolio of Western Blotting primary and secondary antibodies and protein markers, this essential analytical technique is more reliable and convenient than ever before.

The workflow that works

Membrane preparation

Electrophoretically transfer separated proteins onto PVDF or nitrocellulose membrane

Our solution

Protran™ Nitrocellulose

Membrane blocking

Block nonspecific binding sites by incubating the membrane in the blocking buffer and wash

Antibody incubation

- Incubate the membrane with the primary antibody and wash
- Incubate the membrane with the secondary antibody and wash

Our solution

- Primary antibodies*
- Secondary antibodies
  - Horseradish peroxidase (HRP) conjugates
  - Alkaline phosphatase (AP) conjugates

Detection & visualization

Incubate the membrane with appropriate substrate and prepare blot for imaging

- For chemiluminescence substrates, detect proteins using imager or film
- See chromogenic blots with the naked eye or imaging device

Our solution

- Chemiluminescent substrates
  - Western Lightning™
  - Western Lightning™ ONE
- Chromogenic substates
- Prime-Step™ prestained broad-range protein ladder*

Stripping & reprobing

(optional: for chemiluminescent detection only)

- Incubate with stripping buffer and wash
- Incubate with chemiluminescent substrate
- Expose to film or imager to make sure the original signal is removed
- Go back to the blocking step

* Available from BioLegend, a Revvity Company

Transfer membranes: move your detection in the right direction

After gel electrophoresis, separated proteins are transferred from the gel onto a membrane, again by electrophoresis. The membrane is then blocked with neutral proteins, such as BSA or milk, to prevent nonspecific binding of antibodies to the surface of the membrane. The level of sensitivity is influenced by the membrane-binding capacity and relative amount of nonspecific binding.

Explore Protran™, our high-quality pure nitrocellulose transfer membranes for protein binding. A trusted name in detection, it's easy to use and doesn't require a methanol prewetting step. Our transfer membranes provide superior sensitivity, high resolution, and extremely low background.

Features and benefits include:

- 100% nitrocellulose: no cellulose acetate added, ensuring high-binding capacity (80-100 mg/cm^2)
- Low background: top-quality surface properties guarantee exceptional signal-to-noise ratios
- High retention of proteins:
  - 0.2 µm pore size membrane ensures high retention of small proteins below 20 kDa
  - 0.45 µm membrane is ideal for larger molecular weight samples
  - Easy to use, with no methanol prewetting step prior to transfer

Western Blotting transfer membranes	Size	Product description	Part number
Protran™ nitrocellulose transfer membranes	0.2 µm pore size	30 cm x 3 m Roll	NBA083C001EA
		1 Sheet, 15 cm x 15 cm	NBA083S001EA
		5 Sheets, 33 cm x 56 cm	NBA083G001EA
	0.45 µm pore size	15 cm x 3 m Roll	NBA085A001EA
		20 cm x 3 m Roll	NBA085B001EA
		30 cm x 3.5 m/ Roll	NBA085C001EA
		1 Sheet, 15 cm x 15 cm	NBA085S001EA
		5 Sheets, 33 cm x 56 cm	NBA085G001EA

Secondary antibodies: go further with the right detection method

Choosing the right conjugate – dye-labeled, hapten-labeled, or enzyme-labeled secondary antibodies – will depend on the intended protein detection method and will present different advantages and considerations.

The chemiluminescence method

In chemiluminescence detection, the reaction between the reporter enzyme and the substrate releases light, indicating the presence of the target protein. For western blotting, horseradish peroxidase (HRP) and alkaline phosphatase (AP) are the most commonly used reporter enzymes conjugated to secondary antibodies. The chemiluminescent signal decays quickly on the membrane as the substrate is exhausted, but this reaction speed can also be advantageous, allowing for shorter exposure times when imaging the signal.

In addition, this method offers very high sensitivity, detecting protein levels as low as the single-digit pico range for HRP-conjugates, and the low double-digit pico range for AP-conjugates. Using streptavidin-labeled conjugates can amplify the signal for both HRP and AP in many immunoassays including western blot and ELISA with chemiluminescence as well as chromogenic substrates.

The chromogenic method

Chromogenic detection also uses an enzyme-substrate reaction, with secondary antibodies conjugated to either HRP or AP. However, in this method, a chromogenic substrate reacts with the enzymes to produce a colored product that signals the target protein. This resulting signal does not require special imaging instrumentation to visualize, but can be seen by eye, making this detection method very economical. Though not suitable for detecting very low levels of target protein, the chromogenic signal is stable on the blot and can be stored for months without fading.

The fluorescence method

Detection of fluorescent dye-labeled secondary antibodies requires fluorescence-enabled instrumentation and offers several advantages, including the ability to multiplex or detect multiple proteins of interest on the same blot.

See our portfolio of HRP conjugates

See our portfolio of AP conjugates

View our table of secondary antibodies

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Our HRP- and AP-linked secondary antibodies for Western Blotting

	HRP				AP	
Target	**Goat**	**Mouse**	**Donkey**	***Conjugate***	**Goat**	***Conjugate***
Anti-mouse	NEF822001EA 405306 ‡				NEF824001EA	
Anti-human	NEF802001EA					
Anti-rabbit	NEF812001EA* NEF812E001EA	410406 ‡	406401 ‡		NEF814001EA	
Anti-fluorescein				NEF710001EA		NEF709001PK**
Anit-dgoxiginen		NEF832001EA				

1 mg at 1 mg/mL unless otherwise noted; For products with ‡, see BioLegend website for details.

* 0.1mg lyophilized

** 2 x 275 µL vials

‡ Available from BioLegend, a Revvity Company

HRP conjugates detectable by chemiluminescent or chromogenic substrates

HRP is a 44 kDa glycoprotein that catalyzes the oxidation of specific substrates when hydrogen peroxide is present. In chemiluminescent detection, this results in the emission of light; in chromogenic detection, it results in the deposition of colored ionic reactions, signaling the presence of the protein of interest.

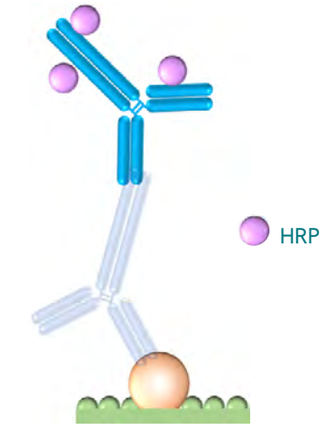
Conjugated to antibodies or streptavidin, HRP is widely used for detecting specific molecular targets in western blotting and other protein detection methods. The increased turnover rate of HRP allows you to quickly achieve a high signal. Providing excellent stability, HRP is the most popular enzyme for chemiluminescent western blotting.

Our HRP-conjugated secondary antibodies are:

- Affinity-purified polyclonal antibodies against mouse, rabbit, or human immunoglobulin G (IgG) heavy and light chains (whole IgG) made in goat and labeled with horseradish peroxide
- Tested to ensure specificity and lot-to-lot consistency
- Provided in liquid form at 1 mg at 1 mg/mL (unless otherwise noted)

Our HRP-conjugated secondary antibodies

Product description	Part number
Anti-rabbit IgG (goat), HRP-labeled	NEF812001EA
Anti-rabbit IgG (goat), HRP-labeled (0.1mg lyophilized)	NEF812E001EA
Anti-mouse IgG (goat), HRP-labeled	NEF822001EA
Anti-human IgG (goat), HRP-labeled	NEF802001EA
Anti-fluorescein-HRP conjugate	NEF710001EA
Anti-digoxigenin (mouse) HRP conjugate	NEF832001EA



Secondary Ab labeled with HRP

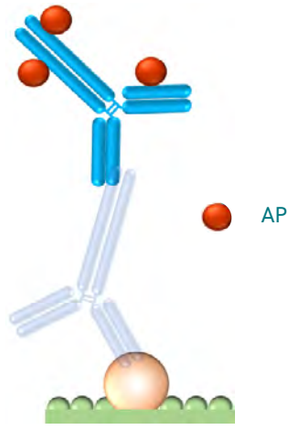
AP conjugates detectable by chemiluminescent or chromogenic substrates

AP is a 140 kDa dimeric metalloenzyme that catalyzes the removal of phosphate. When paired with the appropriate substrate, the reaction causes either emission of light for chemiluminescent detection or dye deposition (BCIP-NBT) for chromogenic detection.

More thermally stable than HRP, AP offers high sensitivity and long signal life in chemiluminescence.

Our AP-conjugated secondary antibodies are:

- Affinity-purified polyclonal antibody against mouse or rabbit IgG heavy and light chains (whole IgG) made in goat and labeled with phosphatase
- Advanced conjugation technology offers three times higher sensitivity than standard products
- Stable for minimum of one year when stored at 2-8°C
- Tested to ensure specificity and lot-to-lot consistency
- Provided in liquid form at 1 mg at 1 mg/mL (unless otherwise noted)



Secondary Ab labeled with AP

Our AP-Conjugated Secondary Antibodies

Product description	Part number
Anti-rabbit IgG (goat), AP conjugate	NEF814001EA
Anti-mouse IgG (goat), AP conjugate	NEF824001EA
Anti-fluorescein-AP conjugate (2 x 275 µL vials)	NEF709001PK

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Chemiluminescent substrates: driving detection with lightning performance

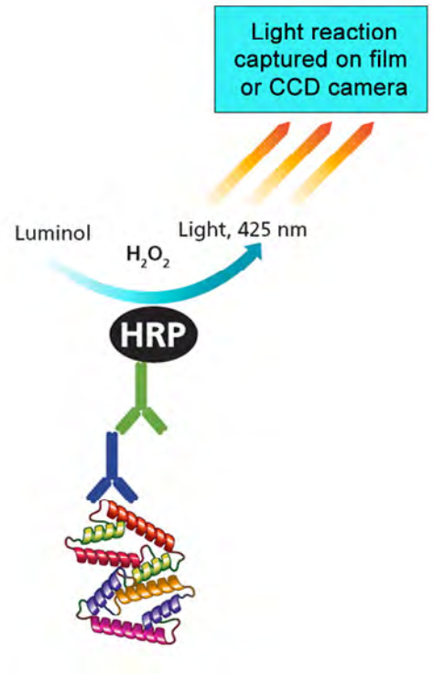
Chemiluminescent substrates are important tools in the detection of proteins. Essentially, chemiluminescence blot detection starts when the primary antibody recognizes and binds to the target protein immobilized on a membrane. This is followed by a secondary antibody labeled with an enzyme such as HRP or AP. A chemiluminescent substrate for that particular enzyme is then applied to the blot causing a light-emitting reaction and signaling the presence of the target.

The most popular choice in detection methods, our Western Lightning™ chemiluminescent substrates combine exceptional sensitivity and dynamic range with safe, enhanced luminol chemistry. The signal is transient and decays within hours after substrate exhaustion.

We offer a full range of superior products to meet your specific performance and budget needs.

Discover our Western Lightning Chemiluminescent substrates

View Western Lightning ONE competitor comparison

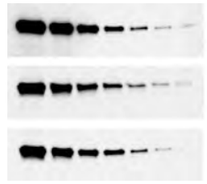
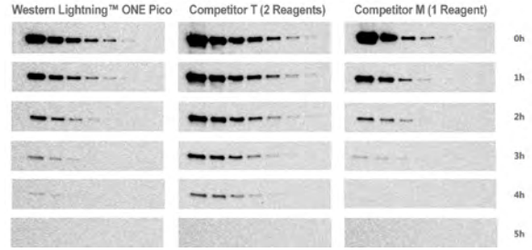
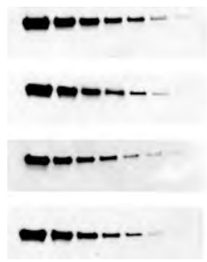
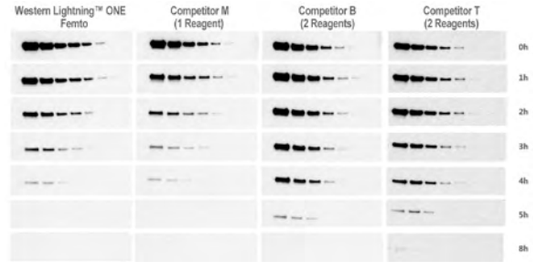
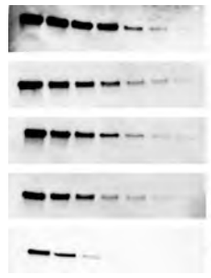
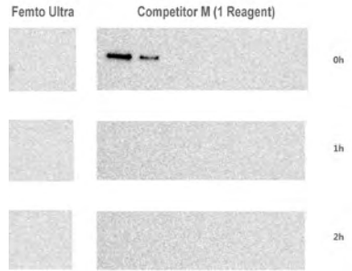


Product	Description	Unit size	Part number
Western Lightning ONE - Ready-to-use, premixed single component			
Western Lightning ONE Pico	▪ Mid-picogram detection ▪ Ideal for most routine analysis of abundant proteins ▪ Low background for high signal to noise ratio	25 mL	NEL130001EA
		250 mL	NEL131001EA
		500 mL	NEL132001EA
Western Lightning ONE Femto	▪ Mid-femtogram detection ▪ Well-suited for a wide range of antibody dilutions ▪ Detects protein levels with high signal intensity	25 mL	NEL140001EA
		250 mL	NEL141001EA
		50 0mL	NEL142001EA
Western Lightning ONE Femto Ultra	▪ Low femtogram detection ▪ Low antibody consumption ▪ Detects protein levels with superior signal intensity	25 mL	NEL150001EA
		125 mL	NEL151001EA
		250 mL	NEL152001EA
Western Lightning - Two-component substrates that require mixing and offer protein detection at varying sensitivities to fit your needs			
Western Lightning Plus	▪ Detects proteins in 1-10 picogram range ▪ Reduces cost of assay by using fewer primary and secondary antibodies ▪ Works well with PVDF and nitrocellulose membranes	30 mL	NEL103E001EA
		130 mL	NEL103001EA
		340 mL	NEL104001EA
		680 mL	NEL105001EA
Western Lightning Pro	▪ Detects proteins in low picogram ranges ▪ Delivers outstanding value ▪ Offers easy conversion ▪ Works well with PVDF and nitrocellulose membranes	30 mL	NEL120E001EA
		130 mL	NEL120001EA
		340 mL	NEL121001EA
		680 mL	NEL122001EA
Western Lightning Ultra	▪ Detects proteins in low femtogram range ▪ Uses less of your primary antibody and sample ▪ Wide dynamic range, for robust results in just one experiment ▪ Immediate, intense signal for at least eight hours; ideal for CCD imagers as well as film ▪ Works well with PVDF and nitrocellulose membranes	20 mL	NEL111001EA
		110 mL	NEL112001EA
		220 mL	NEL113001EA

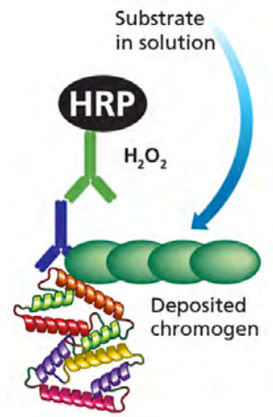
Comparison of Western Lightning **ONE** versus major competitors:

Performance and signal duration

Western blotting detection of HDAC-1 in HeLa cell lysate.
Exposure time is 120 secs for each time point.

Performance	Signal duration
Detection level: Mid-picogram	
	
Detection level: Mid-femtogram	
	
Detection level: Low femtogram	
	

Chromogenic substrates: colorimetric detection that stands the test of time



Chromogenic detection gives you the convenience of direct colorimetric visualization of results without the need for film or imaging instrumentation. With permanent results recorded on a transfer membrane that won't fade over time, chromogenic substrates provide an easy and cost-effective technique for western blotting.

Our chromogenic substrates

Detection of HRP

Product	Description	Unit size	Part number
4CN *Plus*	▪ Detection of HRP, for up to 3,000 cm2 of membrane ▪ 10 times more sensitive than standard 4CN (4-chloro-1-napthol) formulations ▪ Produces dark purple precipitate in the presence of HRP	2 bottles	NEL300001EA
DAB Substrate Solution	For detection of HRP in blotting and slide applications	10 mL	NEL938001EA

Detection of AP

Product	Description	Unit size	Part number
BCIP/NBT	▪ Detection of phosphatase, for up to 2,000 cm2 of membrane ▪ Combination of BCIP (5-bromo-4-chloro-3- indolyl-phosphate) and NBT (nitroblue tetrazolium) produces much higher sensitivity than either reagent separately ▪ Deposits a permanent dark purple stain on membrane sites bearing phosphatase	2 x 250 mL	NEL937001PK

View a side-by-side comparison between 4CN Plus and BCIP/NBT detection methods

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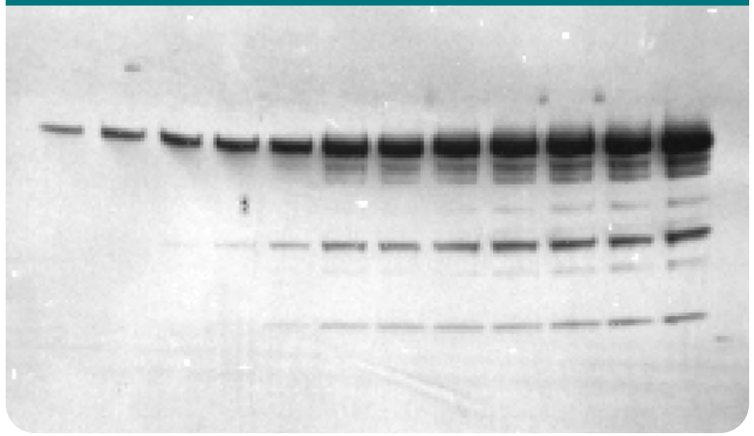
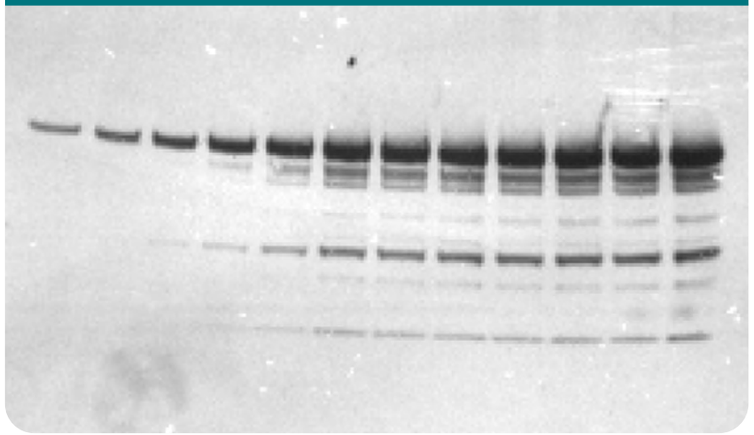
Comparison of 4CN Plus and BCIP/NBT detection methods

4CN Plus imaged



4CN Plus substrate detects HRP and is ideal for western blotting applications.

BCIP/NBT imaged



BCIP/NBT substrate detects AP in blotting and slide applications.

Dilutions of bovine □-tubulin (starting at 800 ng) were electrophoresed and electroblotted onto PolyScreen PVDF transfer membrane. Western blot detection was carried out using anti-tubulin antibody, either goat anti-mouse HRP or goat anti-mouse AP. Stained blots were visualized on a commercially available imaging system.

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The platform that keeps your research rolling full steam ahead

Western blotting is an analytical technique that's fundamental to protein research. Its contributions to science are wide ranging, from neuroscience to infectious disease to drug discovery and beyond. Our western blotting solutions give you the confidence you need to keep your research moving forward.

Click the links for examples of how scientists have used our western blotting solutions to advance their research.

Gene editing

Learn how western blotting is used in molecular biology to validate gene insertion and knockout in CRISPR and other gene editing applications.

View app note

Oncology

Assess the role of proteins in cancer and aid the development of protein-based therapeutics.

Read article

Infectious diseases

Evaluate proteins that contribute to drug resistance and help characterize vaccine candidates.

Read article

Neuroscience

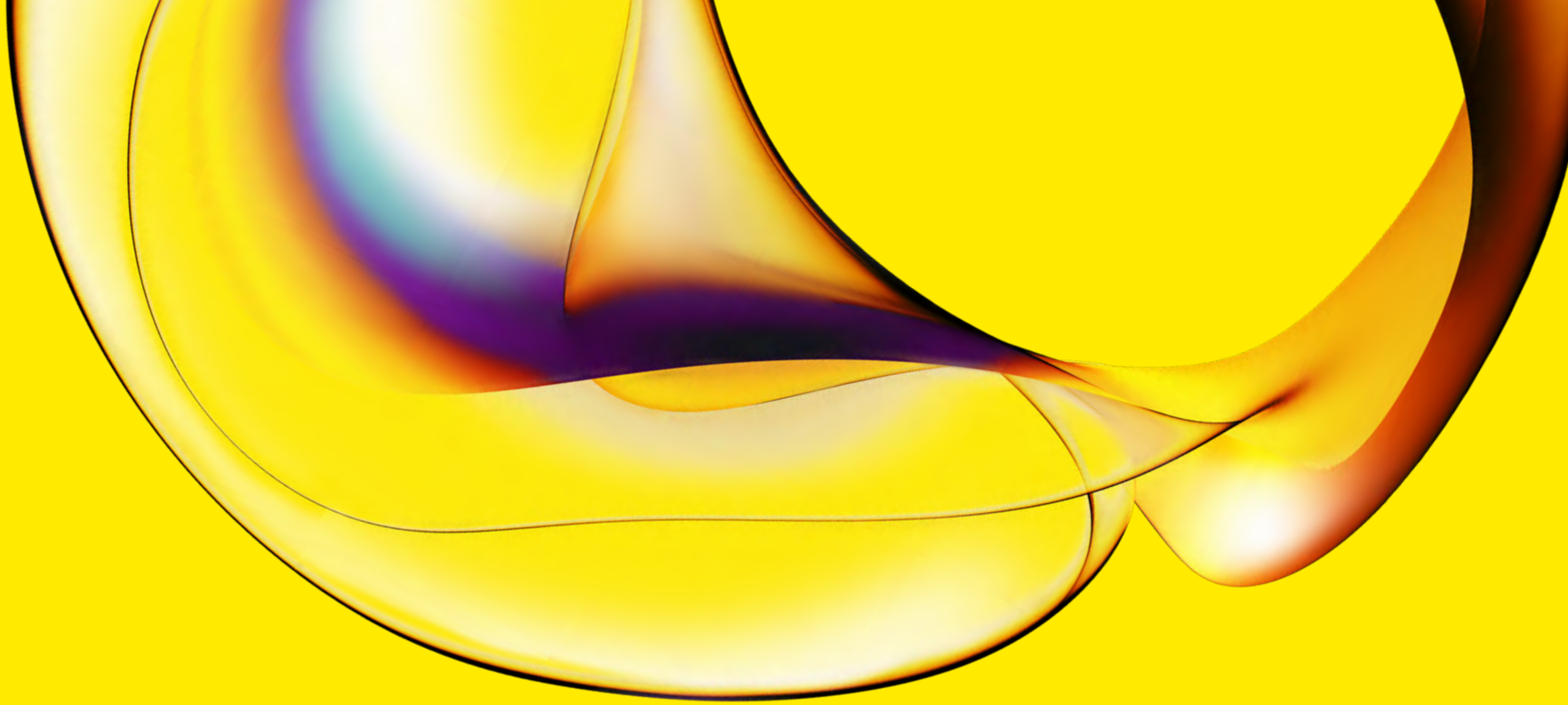
Gain a deeper understanding of proteins involved in neurological and neurodegenerative diseases.

Read article

Drug discovery & development

Support drug candidate characterization, optimization, and validation of drug efficacy and toxicity.

Read article



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