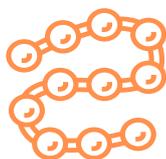


ElioDX™ Do-it-yourself (DIY) Kit

Description ElioDX™ assays are immunoassays based on evanescence for the *in vitro* quantitative analysis of analytes. ElioDX™ technology serves a broad spectrum of needs across **research, diagnostics, and pharmaceutical development**, offering key advantages in flexibility, speed, and accessibility. The ElioDX™ system is compatible with a broad range of analytes, including **antibodies, proteins, peptides, nucleic acids, and small molecules such as drugs**. This versatility makes it suitable for both research and diagnostic applications.



Antibody



Protein

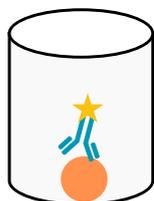


Nucleotide

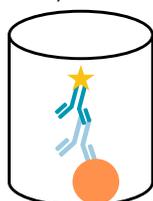


Drug

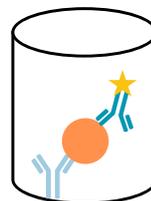
The platform supports multiple immunoassay formats commonly used in ELISA, including **direct, indirect, sandwich, and competitive** assays. This flexibility allows users to tailor assay design to their specific targets and experimental needs, whether for high-affinity binding detection, concentration quantification, or kinetic analysis.



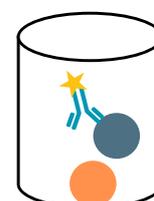
Direct



Indirect



Sandwich



Competitive

What sets ElioDX™ apart is its integration of **evanescent field excitation, real-time fluorescence monitoring, and photon-counting detection**, delivering faster results, higher sensitivity, and lower background compared to conventional ELISA platforms. This combination offers researchers the flexibility of standard assay designs with the performance advantages of next-generation detection technology.

Intended use The ElioDX™ assays are intended for use on ElioDX™ Readers in combination with software provided by ELIONOVA AG. For research use only. Not for use in diagnostic procedures.

Key features EVA technology by ELIONOVA works by generating an evanescent field of exciting light at the bottom of the well. The exciting laser light beam (635nm) is totally reflected at the liquid - solid interface. A 200 nm bottom layer of the adjacent liquid is selectively illuminated: only fluorophores localized in this evanescent field absorb the laser beam photons and subsequently emit light of longer wavelength. The liquid above the 200nm range is not illuminated. An analyte captured at the bottom of the well is detected by an antibody labelled with a fluorophore, which now can absorb light and then emit light of a longer wavelength. The emitted light leaving the device through the bottom is recorded in real time. Fluorophores not being bound to the bottom within the evanescent field (as the specific analyte of interest is not present) do not create a signal (Figure 1).

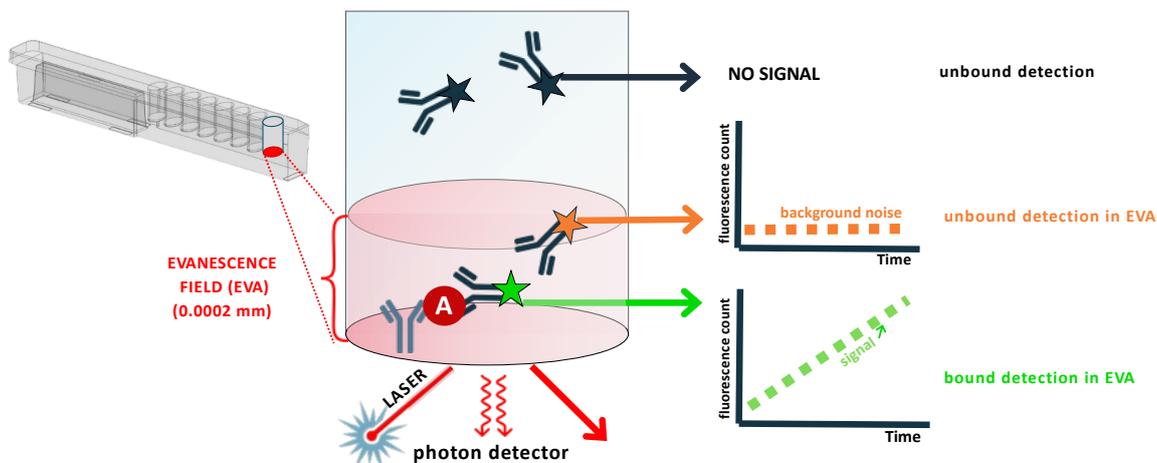


Figure 1. The ElioDX™ system uses evanescent field (EVA) technology for specific fluorescence detection. Detection antibodies labeled with fluorophores bound to the analyte generate signals (green star) within the evanescent field. Unbound antibodies cause low background fluorescence (orange star), while fluorophores outside the field produce no signal (black star). This reduces background and enables sensitive, real-time quantification.

General Procedure

Elionova enables the users to make their own assays. ELIONOVA supports users with protocols and reagents in developing own custom assays using the ElioDX™ platform. The process is straightforward, as it relies on familiar materials and conditions. For example, the assay format uses standard high-binding polystyrene surfaces, ensuring reliable molecule immobilization. The reaction kinetics are preserved thanks to the flat-bottom reaction vessel design, which promotes uniform mixing and consistent signal development. Users can work with buffer systems and temperature conditions, like those used in ELISA, making assay transfer and optimization both efficient and predictable.

We provide a flexible immunoassay development workflow that follows standard steps:
Coat → Block → Stabilize → Add Detection → Measure.

While the underlying protocol remains consistent, we offer flexible assay formats and support to enable customers to develop and run their own assays that suit different research workflows and development stages (Figure 2).

Wet-Wet Format

- All steps are performed wet, without drying.
- Ideal for **rapid assay validation and optimization.**
- **Not storable**, but allows quick troubleshooting and method setup.
- Before measurement, **sample and detection antibody** must be added.

Dry-Wet Format

- Capture and blocking reagents are dried; detection antibody is added wet.
- Allows **long-term storage.**
- Suitable for labs building toward an optimized, storable format.
- **Sample and detection antibody** are added before measurement.

Dry-Dry Format

- Capture and detection antibodies are both dried in the well.
- Ideal for **fully prepared, ready-to-use assays.**
- Requires **only sample addition**—a true single-step protocol.
- **Highly storable** and convenient for routine use or field deployment.

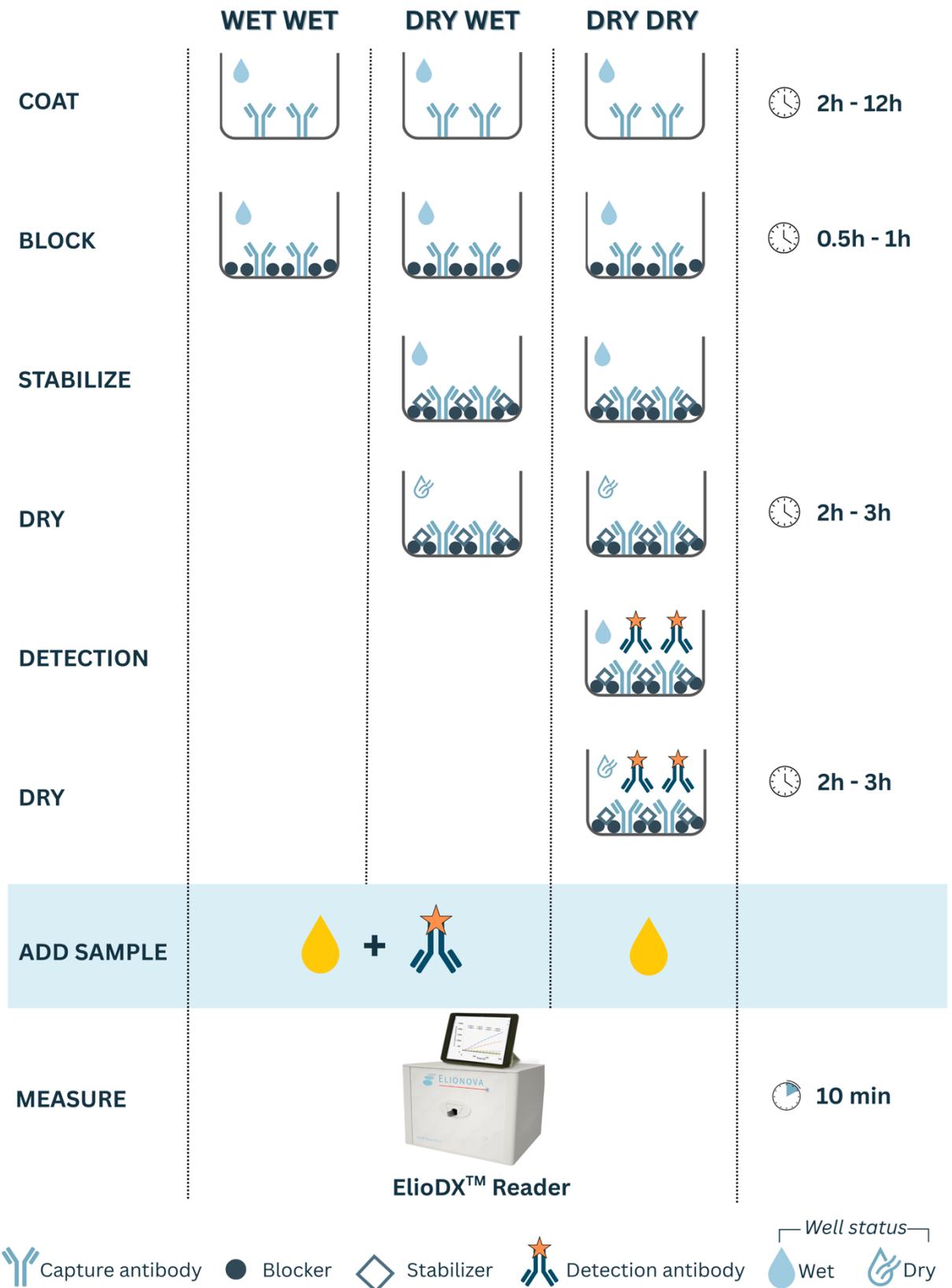


Figure 2. Flexible immunoassay preparation formats supported by Elionova. Three flexible formats—Wet-Wet, Dry-Wet, and Dry-Dry—allow users to adapt the immunoassay workflow for rapid testing or long-term storage.

Contents and Storage

Components	Quantity	Storage
ElioDX™ Core Cuvette	100 units	Room Temperature
ElioDX™ 10X Coating Buffer pH 7.4	1 x 10 ml	2-8 °C
ElioDX™ 10X Washing Buffer	2 x 10 ml	2-8 °C
ElioDX™ 10X Blocking Buffer	1 x 10 ml	2-8 °C
ElioDX™ 10X Stabilizing Buffer	1 x 10 ml	2-8 °C
ElioDX™ 10X Detection Buffer	2 x 1 ml	- 20 °C
ElioDX™ 5X Assay Diluent	1 x 5 ml	2-8 °C

Protocol

Refer to **ElioDX™ Do-it-yourself (DIY) Kit - Cuvette Preparation Appendix** for detailed tables on calculating the required volumes of buffers and reagents for each step, and for instructions on preparing 1X solutions from 10x stock.

Step 1. Coating (see *Cuvette Preparation Appendix, Tables 1, 4, 5*)

1. Add **30 µL** of capture antibody (diluted in ElioDX™ 1X Coating Buffer pH 7.4) to each well of an 8-well cuvette.
2. Incubate at **2–8 °C overnight (12–16 h)** in a humidified chamber.

Step 2. Washing (see *Cuvette Preparation Appendix, Table 6*)

1. Remove unbound capture antibody by thoroughly aspirating or tapping out the contents.
2. Wash each well **four times** with **40–60 µL** ElioDX™ 1X Washing Buffer using a pipette or squeeze bottle.
3. After each wash, thoroughly aspirate or tap out the contents.

Step 3. Blocking (see *Cuvette Preparation Appendix, Table 2, 7*)

1. Add **40 µL** ElioDX™ 1X Blocking Buffer to each well.
2. Incubate at **room temperature (RT)** for **1 hour**.
3. At this point, choose one of the following:
 - **Option A:** Continue directly to **Step 5** and **Step 7** for *wet–wet cuvettes*.
 - **Option B:** Proceed to **Step 4** for *dry–wet cuvettes*.
 - **Option C:** Proceed to **Steps 4, 5, and 6** for *dry–dry cuvettes*.

Step 4 – Stabilization (for dry–wet or dry–dry cuvettes) (see *Cuvette Preparation Appendix, Tables 2, 8*)

1. Remove ElioDX™ 1X Blocking Buffer.
2. Wash each well **two times** with **40–60 µL** ElioDX™ 1X Washing Buffer and **one time** with 40–60 µL ddH₂O.
3. Add **40 µL** ElioDX™ 1X Stabilizing Buffer, incubate **15 min at RT**, then tap out.
4. After each wash, **thoroughly aspirate or tap out contents** onto tissue paper with firm tapping. Clean outside of the 2 sides and the backside of cuvettes with absorbent paper.
5. Put the cuvettes at **37 °C** into an incubator for **2–3 h**.
6. Remove the cuvettes from incubator, place in a **plastic air-lock box** at **RT (15–25 °C)** for **30 min**.

7. After this step, cuvettes can be used immediately or stored at **2–8 °C** in an airtight box as *dry–wet cuvettes*.

Step 5 – 10X Detection Mix Preparation (see *Cuvette Preparation Appendix, Tables 3, 9*)

Prepare the **10X Detection Mix** according to the recipe in **ElioDX™ Do-it-yourself (DIY) Kit - Cuvette Preparation Appendix Table 9, “Preparation of 10X Detection Mix of fluorescent-labeled antibody at 50 µg/ml”**

1. Refer to Cuvette Preparation Appendix Tables 3 and 9 for the exact volumes of:
 - ElioDX™ 10X Detection Buffer
 - ddH₂O
 - 10X fluorescent-labeled antibody
2. Scale the quantities proportionally to your required final volume.
3. Mix gently by pipetting up and down — **avoid foaming**.
4. Use freshly prepared detection mix for optimal performance.

Step 6. Detection Mix coating (for dry dry cuvettes)

1. After Step 4 drying, add **2 µL 10X ElioDX™ Detection Mix** (containing fluorescent-labelled antibody) to each well.
2. Dry completely at **37 °C** for **2-3 h**.
3. Remove from incubator, place in a **plastic air-lock box** at **RT (15–25 °C)** for **30 min**.
4. After this step, cuvettes can be used immediately or stored at **2–8 °C** in an airtight box as *dry–dry cuvettes*.

Step 7: Prepare Sample and Measurement

For wet–wet and dry–wet cuvettes:

1. Mix sample with 10X Detection Mix in a **9:1 ratio**.
2. Add **20 µL** of the sample–detection mix to each well of the Cloud-registered cuvette (see ElioDX™ Cloud instructions).
3. Clean outside and bottom of cuvette with a clean tissue (e.g., Kimberly-Clark™ ref. 7552 or equivalent).
4. Insert into the ElioDX™ Reader and measure according to the operating instructions.

For dry–dry cuvettes:

1. Add **20 µL sample** to each well of the Cloud-registered cuvette.
2. Shake using Vortex-Genie 2 (ref. G560E) in the Elionova AG cuvette shaking holder, speed **2**, for **30 s**.
3. Clean cuvette exterior as above.
4. Measure according to the reader's instructions.

NOTE:

- Bring all reagents, samples and prepared cuvettes to room temperature before use.
- Unused prepared cuvettes must remain sealed and should be kept at cold storage (2°C to 8°C)

- The time from adding the first sample to starting the measurement should **not exceed 2 minutes**.

Precautions

- Do not touch the sides of the cuvette located near the wells with your fingers, as this may negatively impact optical measurement.
- We recommend that this product is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory coats and gloves. Do not pipet by mouth. Do not eat, drink or smoke in areas where the kit and samples are handled.
- Avoid contact of skin or mucous membranes with samples and with samples dissolved in the kit.
- Do not use kit reagents beyond expiry date on label.
- The kit is intended for research use only and not for use in diagnostic or therapeutic procedures.

Stability

Biofunctionalized cuvettes are stable at 2°C to 8°C up to 1 year.

Ready-to-use cuvettes containing dried detection reagents are stable at 2°C to 8°C up to 1 year.

Materials required not provided

The following materials are not included in the kit, but will be required to perform the assay:

- ElioDX™ Reader
- Sterile distilled H₂O
- 0.5 µL to 1000 µL adjustable single channel or multichannel micropipettes with disposable tips



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