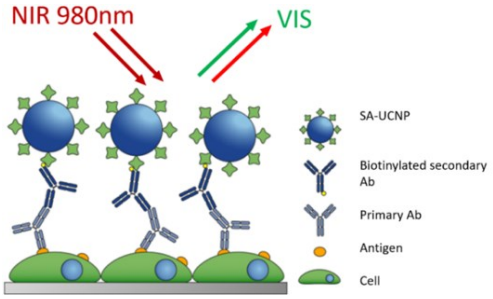
INSTRUCTIONS FOR USE

SCIZYS Erbium Kit

Intended Use

SCIZYS Erbium Kit is an immunohistochemical detection kit based on upconversion nanoparticles (UCNP). The UCNPs are designed to label biotinylated affinity reagents, such as primary or secondary antibodies, in formalin-fixed paraffin embedded (FFPE) sections. For imaging and contextual interpretation, the section is to be counterstained with haematoxylin.

SCIZYS Erbium Kit is aimed for use within a standard laboratory environment by laboratory personnel. The UCNP labelled samples are to be scanned using SCIZYS scanner, which captures images of biological samples on standard histology glass slides, using transmitted brightfield light as well as upconversion fluorescence light. SCIZYS Erbium Kit is intended for research use only - not directly, or indirectly, for *in vitro* diagnostic procedures.



Labelling Scheme

When bound to the secondary antibody, the UCNPs in SCIZYS Erbium-SA visualises the antigens labelling obtained by the primary antibody. Visualisation takes place when near-infrared light (NIR) from the LUMITO SCIZYS scanner excites the UNCPs, which then emit visible light. This anti-Stokes effect significantly reduces the optical background signal by completely avoiding autofluorescence.

SCIZYS Erbium Kit (SZERKIT-01-01) Components

Product	Article no.	Contents
SCIZYS Erbium-SA	SZERSA-01-01	0.75 mg (5–10 mg/mL); vol. 75–150 µL
SCIZYS Dilution Buffer	SZDB-01-01	50 mL
SCIZYS Wash Buffer (20×)	SZWB-01-01	500 mL

SCIZYS Erbium-SA diluted to 50 µg/mL, is sufficient for labelling of 50 sections (300 µL/section). The SCIZYS Wash Buffer (20×) can effectively yield 10 Liters of 1× wash buffer and is suitable for all washing steps included in the immunohistochemical (IHC) workflow.

Reagent Storage and Stability

- SCIZYS Erbium-SA can be handled under ambient light.
- Store SCIZYS Erbium-SA and SCIZYS Dilution Buffer at +2°C – +8°C; do not freeze.
- Store SCIZYS Wash Buffer (20×) at room temperature.
- After use, the reagents must be stored under the recommended storage condition.
- The expiration date is valid only if the components of the SCIZYS Erbium Kit are stored under the recommended storage condition.

Materials Not Provided

- Deparaffinization reagents
- Ethanol
- Heat-induced epitope retrieval (HIER) reagents
- Haematoxylin
- Biotin-free blocking agent
- Streptavidin (SA), and biotin solutions used for blocking
- Primary antibody and biotinylated secondary antibody
- Mounting Medium

Cautions

- Do not use nail polish in combination with SCIZYS Erbium Kit and scanner. Exposure to NIR light in the SCIZYS scanner can cause damage to the section and/or scanner due to certain ingredients in nail polish.
- Ensure that the slide label is applied in its dedicated area of the slide.
- Make sure that the slide is clean before inserting it into the SCIZYS Scanner.

IHC Labelling Procedure

A general protocol applicable for labelling with SCIZYS Erbium is provided in this document. Detailed protocols for different markers tested by Lumito can be found under the following link: www.lumito.se/en/for-users/. Include applicable positive and negative tissue controls while performing IHC protocols.

1. Sectioning

→ Prepare tissue/cell sections with a thickness of 3–4 µm and place them on IHC glasses.
→ If necessary, bake the slides at 60 °C before starting the labelling procedure.

2. Deparaffinization and Rehydration

→ For FFPE sections, perform the deparaffinization and rehydration steps according to standard procedures.

3. Epitope Retrieval

→ Perform the epitope retrieval method (HIER or enzymatic) appropriate for the primary antibody used.

4. SCIZYS Wash Buffer (20×) Dilution

→ For example, to prepare 1 L of wash buffer, add 50 mL of SCIZYS Wash Buffer (20×) to 950 mL of deionized water.
→ Mix the diluted SCIZYS Wash Buffer (20×) gently to prevent excessive foam formation.

5. Haematoxylin Counterstaining

→ **IMPORTANT:** Perform haematoxylin counterstaining after epitope retrieval and prior to the blocking step by incubating the slides for 30 s to 3 min in Mayer's Haematoxylin.
→ Wash slides with deionized water until the water appears clear.
→ Transfer the slides into wash buffer.

6. Blocking of Non-specific Binding Sites and Endogenous Biotin

→ Create a hydrophobic barrier around the sections with a PAP pen.
→ Block non-specific binding sites using a biotin-free blocking agent.
→ Wash the slides once with wash buffer.
→ Block endogenous biotin with a biotin blocking kit according to the manufacturer's instructions.
→ Wash the slides twice after each step with wash buffer.

7. Primary Antibody

→ Apply the primary antibody according to the manufacturer's instructions.
→ Remove excess primary antibody by washing the slides twice in wash buffer.

8. Biotinylated Secondary Antibody

→ Add a biotinylated secondary antibody raised against the species of the primary antibody and incubate for 30–60 minutes.
→ Remove excess biotinylated secondary antibody by washing the slides twice in wash buffer.

9. SCIZYS Erbium-SA

→ Gently mix SCIZYS Erbium-SA before use. (To prevent loss of sample that may be stuck in the vial cap, it is advisable to vortex mix, swing and gently tap the vial on the lab bench before opening.)
→ Dilute SCIZYS Erbium-SA in SCIZYS Dilution Buffer to a final concentration of 10–50 µg/mL. The optimal concentration depends on the experiment performed. At a concentration of 50 µg/mL in SCIZYS Dilution Buffer, the SCIZYS Erbium-SA particles remain stable for at least 6 hours.
→ Samples labelled with SCIZYS Erbium-SA can be handled under ambient light.
→ Apply SCIZYS Erbium-SA onto the slides and incubate for 30–60 minutes.
→ Store the reagents at +2 °C to +8 °C after use.
→ Wash the slides three times with wash buffer.

10. Mounting

→ Mount the slides with mounting media compatible with immunofluorescence.

Contact Information

Please notify LUMITO AB if this product is received damaged.
Additional information is available on our website: www.lumito.se/en/for-users/
Email: support@lumito.se

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