RAL DIFF-QUIK™ EZ KIT (3x100ml)
RAL DIFF-QUIK™ KIT (3x0,5L)

FAST STAINING KIT

**Principle:**
RAL Diff-Quik is a fast-acting variation of Romanowsky staining. In an aqueous buffered medium, this kit enables:
- A differential staining of blood smears (differential blood cell counting, morphological study of leucocytes, study of parasites) and medullary smears (myelograms).
- The detection of tissue and blood parasites in medical and veterinary mycology.
- The cytological and structural study of fixed and paraffin embedded tissue sections as well as fluids and punctures.
- The cytological study of urines, spinal and other fluids.

The analysis of smears is identical to the one carried out with standard Romanowsky staining.

**Kit Description:** (3 x 100 ml or 3 x 500 ml)

- **Bottle 1:** RAL Diff-Quik® Fixative solution 100 ml or 500 ml
- **Bottle 2:** RAL Diff-Quik® Solution I 100 ml or 500 ml
- **Bottle 3:** RAL Diff-Quik® Solution II 100 ml or 500 ml

**Number of tests possible:** 100 to 200 slides for 3 x 100 ml 800 to 1000 slides for 3 x 500 ml

**Available refills:**
- RAL DIFF-QUIK™ Fixative solution (1x 2,5L) REF : 720560-2500
- RAL DIFF-QUIK™ Solution I (1x 2,5L) REF : 720570-2500
- RAL DIFF-QUIK™ Solution II (1x 2,5L) REF : 720580-2500

**Use:**
For professional use only.
For in-vitro diagnostic use.

The collection and processing of chemical and biological waste must be conducted by specialized and registered companies.

Storage Temperature: 15-25°C away from light.

Shelf-Life after Opening: 2 months (for EZ-kit only, see notes)

**Specimen preparation:**
Specimen must be treated in accordance with procedures available in the laboratory and promulgated by national authorities.

**Sample processing:**

- **Plasmodium:** Thick Blood Smears
  - Length of one Test: 7 seconds
  - Take 2 μl of blood out of an EDTA tube and place them in the center of a slide.
  - Spread the drop in a circle with the corner of another slide.
  - Allow it to dry for 20 minutes in the open air, for 5 minutes in an incubator, or for 2 minutes under a hair dryer.
  - Cover the drop with very little tap water to haemolyse it.
  - Once the haemoglobin has spread, get rid of the red water just inclining the slide.
  - Rinse the drop very gently with tap water.
  - The drop then gets a whitish appearance.

- **Cytology:** Spermocytograms
  - Length of one Test: 1 minute and 20 seconds
  - Dip the slide 1 minute in solution RAL Diff-Quik Fixative (bottle 1).
  - Drain surplus solution onto filter paper.
  - Dip the slide 1 minute in solution RAL Diff-Quik Solution I (bottle 2).
  - Drain surplus solution onto filter paper.
  - Dip the slide 5 x 1 second in solution RAL Diff-Quik Solution II (bottle 3).
  - Rinse the slide with distilled water.

**Recommendations and/or notes of use:**
According to the thickness of the smear, it may be necessary to increase the fixative time (solution 1).

The parietal structures of fungi and cystic forms of Pneumocystis carinii do not stain and appear as a light halo.

In the case of 3 x 100 mL kit, the use of wide-necked bottles allows staining of slides directly in the bottle. This reduces the risk of evaporation and air-oxidation because there is no need of solutions transfer.

The 3 bottles must be stored away from light.

**Bibliography:**

**Staining procedures:**

**Haematology: Blood Smears**
- Length of one Test: 15 seconds
  - Dip the slide 5 x 1 second in RAL Diff-Quik Fixative (bottle 1).
  - Drain surplus solution onto filter paper.
  - Dip the slide 5 x 1 second in solution RAL Diff-Quik Solution I (bottle 2).
  - Drain surplus solution onto filter paper.
  - Dip the slide 5 x 1 second in solution RAL Diff-Quik Solution II (bottle 3).
  - Rinse the slide with distilled water.

**Haematology: Bone marrow**
- Length of one Test: 3 min
  - Dip the slide 1 minute in solution RAL Diff-Quik Fixative (bottle 1).
  - Drain surplus solution onto filter paper.
  - Dip the slide 1 minute in Diff-Quik Solution I (bottle 2).
  - Drain surplus solution onto filter paper.
  - Dip the slide 1 minute in RAL Diff-Quik Solution I (bottle 2).
  - Drain surplus solution onto filter paper.
  - Rinse the slide with distilled water.

**Cytology:**
- Spermocytograms
  - Length of one Test: 1 minute and 20 seconds
  - Dip the slide 1 minute in solution RAL Diff-Quik Fixative (bottle 1).
  - Drain surplus solution onto filter paper.
  - Dip the slide 25 seconds in RAL Diff-Quik Solution I (bottle 2).
  - Drain the excess solution onto filter paper.
  - Dip the slide 25 seconds in RAL Diff-Quik Solution II (bottle 3).
  - Rinse the slide with distilled water.

**Cyto-bacteriology:**
- cytology of biological fluids, urines, cytopunctures and CSF
  - Length of one Test: 15 seconds
  - Dip the slide 5 seconds (1 minute for CSF) in RAL Diff-Quik Fixative (bottle 1).
  - Drain surplus solution onto filter paper.
  - Dip the slide 5 seconds (1 minute for CSF) in RAL Diff-Quik Solution I (bottle 2).
  - Drain surplus solution onto filter paper.
  - Dip the slide 5 seconds (2x1 second for CSF) in RAL Diff-Quik Solution II (bottle 3).
  - Drain surplus solution onto filter paper.
  - Rinse the slide with distilled water.
Cytology of fixed and paraffined tissue sections

**Length of one Test : 13 sec**
- Dewax and hydrate the section
- Dip the slide 5 seconds in RAL Diff-Quik™ Solution I (bottle 1)
- Drain surplus solution onto absorbent paper
- Dip the slide 7 seconds in RAL Diff-Quik™ Solution II (bottle 1)
- Briefly rinse a few seconds
- Shake the slide in 90° Ethanol for 1 to 2 seconds until it gets the wished tint
- Stop the differentiation dipping the slide in Isopropanol
- Mount the slide with Toluene or Xylene based mounting medium.

Parasitology : Plasmodium Thick Blood Smears

**Length of one Test : 7 sec**
- Dip the slide 1 second in RAL Diff-Quik Fixative (bottle 1)
- Drain surplus solution onto absorbent paper
- Dip the slide 3 x 1 second in RAL Diff-Quik™ Solution I (bottle 1)
- Rinse the slide very gently with distilled water
- Dip the slide 3 x 1 second in RAL Diff-Quik™ Solution II (bottle 2)
- Drain surplus solution onto absorbent paper
- Dip the slide 7 seconds in RAL Diff-Quik™ Solution I (bottle 2)
- Drain surplus solution onto absorbent paper
- Dip the slide 5 seconds in RAL Diff-Quik™ Solution I (bottle 2)
- Drain surplus solution onto absorbent paper

Parasitology : Plasmodium Blood Films

**Length of one Test : 1 minute and 4 sec**
- Dip the slide 1 min in RAL Diff-Quik Fixative (bottle 1)
- Drain surplus solution onto absorbent paper
- Dip the slide 2 x 1 second in RAL Diff-Quik™ Solution I (bottle 1)
- Drain surplus solution onto absorbent paper
- Dip the slide 2 x 1 second in RAL Diff-Quik™ Solution II (bottle 1)
- Quickly rinse the slide with distilled water

Parasitology : Tissular Protozoa (Leishmania, Toxoplasma, Microsporidiosis), Cryptosporidium, Pneumocystis carinii, fungi contributing to deep Mycosis

**Length of one Test : 2 minutes and 5 sec**
- Dip the slide 1 second in RAL Diff-Quik Fixative (bottle 1)
- Drain surplus solution onto absorbent paper
- Dip the slide 25 seconds in RAL Diff-Quik™ Solution I (bottle 1)
- Drain surplus solution onto absorbent paper
- Dip the slide 40 seconds in RAL Diff-Quik™ Solution II (bottle 1)
- Quickly rinse the slide with distilled water

Veterinary Parasitology (Pyriplasmosis, M. pachydermatosis)

**Length of one Test : 15 sec**
- Dip the slide 5 x 1 second in RAL Diff-Quik Fixative (bottle 1)
- Drain surplus solution onto absorbent paper
- Dip the slide 5 x 1 second in RAL Diff-Quik™ Solution I (bottle 1)
- Drain surplus solution onto absorbent paper
- Dip the slide 5 x 1 second in RAL Diff-Quik™ Solution II (bottle 1)
- Quickly rinse the slide with distilled water

Trichomonas

**Length of one Test : 2 minutes and 15 sec**
- Dip the slide 15 seconds in RAL Diff-Quik Fixative (bottle 1)
- Drain surplus solution onto absorbent paper
- Dip the slide 1 minute in RAL Diff-Quik™ Solution I (bottle 1)
- Drain surplus solution onto absorbent paper
- Dip the slide 1 minute in RAL Diff-Quik™ Solution II (bottle 1)
- Quickly rinse the slide with distilled water

Microfilaria

**Length of one Test : 30 to 40 sec**
- Dip the slide 10 x 1 second in RAL Diff-Quik Fixative (bottle 1)
- Drain surplus solution onto absorbent paper
- Dip the slide 10 x 1 second in RAL Diff-Quik™ Solution I (bottle 1)
- Drain surplus solution onto absorbent paper
- Dip the slide 10 to 20 x 1 second in RAL Diff-Quik™ Solution II (bottle 1)
- Quickly rinse the slide with distilled water

Histology : Detection of Helicobacter pylori

**Length of one Test : 15 sec**
- Make a smear
- Dip the slide 5 seconds in RAL Diff-Quik Fixative (bottle 1)
- Drain surplus solution onto absorbent paper
- Dip the slide 5 seconds in RAL Diff-Quik™ Solution I (bottle 1)
- Drain surplus solution onto absorbent paper
- Dip the slide 5 seconds in RAL Diff-Quik™ Solution II (bottle 1)
- Drain surplus solution onto absorbent paper
- Stop the differentiation dipping the slide in Absolute Alcohol
- Dip the slides in 2 baths of Xylene or Toluene
- Mount the slide with Toluene or Xylene based mounting medium

Blood smear example:

<table>
<thead>
<tr>
<th>Time</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>5x1 sec</td>
<td>Drain</td>
</tr>
<tr>
<td>5x1 sec</td>
<td>Drain</td>
</tr>
<tr>
<td>5x1 sec</td>
<td>Rinse</td>
</tr>
</tbody>
</table>

Results:

**Blood smear**

- **Nuclei:** Chromatin: ± dense purple
- **Leukocytes:** Cytoplasm without RNA: light pinkish
- **Eosinophilic Granulations:** orange-brown
- **Basophilic Granulations:** dark purple blue
- **Neutrophilic Granulations:** ± intense purple
- **Lymphocytes:** Cytoplasm with RNA: pure blue
- **Cytoplasm without RNA:** light blue
- **Azurophilic Granulations:** red
- **Monocytes:** Cytoplasm: grey-blue
- **Erythrocytes:** light red
- **Platelets:** Chromomere: purple-red
- **Hyalomere:** bluish

**Blood parasites:**
- **Nuclei:** red (plasmodium)
- **Cytoplasm:** blue

**Parasitology and Mycology**

Cytoplasms of host, fungic or parasitic eucaryot cells: blue more or less dark, depending on the ribosomal richness.

Nuclei: purple red

**Helicobacter pylori on histological sections**

- **Helicobacter pylori:** Dark Blue
- **Nuclei:** Blue
- **Cytoplasts:** Pink to red
- **Collagen:** Very pale pink

**Histo-Cyto**

- **Nuclei:** Red violet
- **Cytoplasts:** Pink
- **Basophilic:** Blue
- **Collagen:** Pale pink
- **Erythrocytes:** Beige
RAL DIFF-QUIK™ FIXATIVE SOLUTION - 720560-0100
-0500
-2500

DANGER. H225 Highly flammable liquid and vapour. H311 Toxic in contact with skin. H301 Toxic if swallowed. H370 Causes damage to organs. P202 Do not handle until all safety precautions have been read and understood. P233 Keep container tightly closed. P281 Use personal protective equipment as required. P304+P340 IF INHALED: remove victim to fresh air and keep at rest in a position comfortable for breathing. P309+P311 IF exposed or if you feel unwell. Call a POISON CENTER or doctor/physician.

RAL DIFF-QUIK™ SOLUTION I - 720570-0100
-0500
-2500

WARNING. H317 May cause an allergic skin reaction. P333+P313 If skin irritation or rash occurs: Get medical advice/attention

RAL DIFF-QUIK™ SOLUTION II - 720580-0100
-0500
-2500