

# RAL DIFF-QUIK™ EZ KIT (3x100ml)

## RAL DIFF-QUIK™ KIT (3x0,5L)

REF : 720550-0000

REF : 720555-0000

FAST STAINING KIT

### Principle:

RAL Diff-Quik kit 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 leukocytes, study of parasites) and medullary smears (myelograms)
- The detection of tissular and blood parasites in medical and in veterinary mycology
- The cytological and structural study of fixed and paraffin embedded tissue sections as well as fluids and punctions
- 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. **IVD**

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, or 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.

### 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.



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(Bottle ① contains Methanol which is very hygroscopic, and thus it must therefore be regularly refilled, namely in countries having high humidity). The oxidation level of RAL Diff-Quik™ Solution II is standardised during manufacture.

This level varies over time and in transferring small quantities of RAL Diff-Quik™ Solution I from bottle to bottle . It is essential to drain off excess RAL Diff-Quik™ Solution I (bottle ②) before dipping the slide into RAL Diff-Quik™ Solution II (bottle ③). The colour of RAL Diff-Quik™ Solution I (bottle ②) may appear more or less dark, but the staining results are not affected either way. RAL Diff-Quik™ Solution II (bottle ③) remains stable for 2 months after opening.

### Bibliography:

**DATRY A., LECSO G., RICHARD-LENOBLE D. et KOMBILA M.,** Coloration rapide des plasmodies et des microfilaires par les colorants solubles dans l'eau, Med. Trop., vol 42, n°6, nov-déc 1982, p.673-675.

**JASWANT SINGH, BHATTACHARJI L. M.,** Rapid staining of malarial parasites by a water soluble stain, The Ind. Med. Gaz., n°3, mars 1944, p. 102-104.

**BOURÉE P.,** Aide mémoire de parasitologie et de pathologie tropicale, Médecine-Sciences, Flammarion, 2 ème éd., 1994, p. 294-295.

**PRAT J.J., BROULAND J-Ph., MIKOL J.,** Une alternative à la coloration de Giemsa sur coupe histologique (Service Anatomie et Cytologie Pathologiques - Hôpital Lariboisière), Assises d'Anatomie Pathologiques, 23-24 mars 1955, p. 44.

**BROULAND J.P., PRAT J.J., CASTAGNET P.,** Méthode rapide de coloration pour la mise en évidence de *Helicobacter pylori* (Service Anatomie et Cytologie Pathologiques - Hôpital Lariboisière), Assises d'Anatomie Pathologique, 23-24 mars 1995, p.211.

### Staining procedures:

<b>Haematology: Blood Smears</b> <b>Length of one Test : 15 seconds</b> <ul style="list-style-type: none"> <li>• Dip the slide 5 x 1 second in RAL Diff-Quik Fixative (bottle ①).</li> <li>• Drain surplus solution onto filter paper.</li> <li>• Dip the slide 5 x 1 second in solution RAL Diff-Quik Solution I (bottle ②).</li> <li>• Drain surplus solution onto filter paper.</li> <li>• Dip the slide 5 x 1 second in solution RAL Diff-Quik Solution II (bottle ③).</li> <li>• Rinse the slide with distilled water.</li> </ul>	<b>Haematology: Bone marrow</b> <b>Length of one Test : 3 min</b> <ul style="list-style-type: none"> <li>• Dip the slide 1 minute in solution RAL Diff-Quik Fixative (bottle ①).</li> <li>• Drain surplus solution onto filter paper.</li> <li>• Dip the slide 1 minute in Diff-Quik Solution I (bottle ②).</li> <li>• Drain surplus solution onto filter paper.</li> <li>• Dip the slide 1 minute in RAL Diff-Quik Solution II (bottle ③).</li> <li>• Drain surplus solution onto filter paper.</li> <li>• Rinse the slide with distilled water.</li> </ul>
<b>Cytology: Spermocytograms</b> <b>Length of one Test : 1 minute and 20 seconds</b> <ul style="list-style-type: none"> <li>• Dip the slide 1 minute in solution RAL Diff-Quik Fixative (bottle ①).</li> <li>• Drain surplus solution onto filter paper.</li> <li>• Dip the slide 25 seconds in RAL Diff-Quik Solution I (bottle ②).</li> <li>• Drain the excess solution onto filter paper.</li> <li>• Dip the slide 25 seconds in RAL Diff-Quik Solution II (bottle ③).</li> <li>• Rinse the slide with distilled water.</li> </ul>	<b>Cyto-bacteriology: cytology of biological fluids, urines, cytopunctures and CSF</b> <b>Length of one Test: 15 seconds</b> (1minute 4 seconds for CSF) <ul style="list-style-type: none"> <li>• Dip the slide 5 seconds (1 minute for CSF) in RAL Diff-Quik Fixative (bottle ①).</li> <li>• Drain surplus solution onto filter paper.</li> <li>• Dip the slide 5 seconds (2x1 second for CSF) in RAL Diff-Quik Solution I (bottle ②).</li> <li>• Drain surplus solution onto filter paper.</li> <li>• Dip the slide 5 seconds (2x1 second for CSF) in RAL Diff-Quik Solution II (bottle ③).</li> <li>• Rinse the slide with distilled water.</li> </ul>

**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 ❶).
- Drain surplus solution onto absorbent paper
- Dip the slide 7 seconds in RAL Diff-Quik™ Solution II (bottle ❷).
- 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 Iso-propanol
- 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 ❶)
- Drain surplus solution onto absorbent paper
- Dip the slide 3 x 1 second in RAL Diff-Quik™ Solution I (bottle ❷).
- Rinse the slide very gently with distilled water
- Dip the slide 3 x 1 second in RAL Diff-Quik™ Solution II (bottle ❸).
- Rinse the slide very gently 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 ❶)
- Drain surplus solution onto absorbent paper
- Dip the slide 25 seconds in RAL Diff-Quik™ Solution I (bottle ❷).
- Drain surplus solution onto absorbent paper
- Dip the slide 40 seconds in RAL Diff-Quik™ Solution II (bottle ❸).
- Quickly rinse the slide with distilled water

**Veterinary Parasitology (Piroplasmosis, M. pachydermatis)****Length of one Test : 15 sec**

- Dip the slide 5 x 1 second in RAL Diff-Quik Fixative (bottle ❶)
- Drain surplus solution onto absorbent paper
- Dip the slide 5 x 1 second in RAL Diff-Quik™ Solution I (bottle ❷).
- Drain surplus solution onto absorbent paper
- Dip the slide 5 x 1 second in RAL Diff-Quik™ Solution II (bottle ❸).
- 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 ❶)
- Drain surplus solution onto absorbent paper
- Dip the slide 10 x 1 second in RAL Diff-Quik™ Solution I (bottle ❷).
- Drain surplus solution onto absorbent paper
- Dip the slide 10 to 20 x 1 second in RAL Diff-Quik™ Solution II (bottle ❸).
- Quickly rinse the slide with distilled water

**Cytology of punctions (breast and deep organs), effusion liquids of the serous membrane (pleura, peritoneum ...)****Length of one Test : 15 sec**

- Make a smear
- Dip the slide 5 seconds in RAL Diff-Quik Fixative (bottle ❶).
- Drain surplus solution onto absorbent paper
- Dip the slide 5 seconds in RAL Diff-Quik™ Solution I (bottle ❷).
- Drain surplus solution onto absorbent paper
- Dip the slide 5 seconds in RAL Diff-Quik™ Solution II (bottle ❸).
- 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 ❶)
- Drain surplus solution onto absorbent paper
- Dip the slide 2 x 1 second in RAL Diff-Quik™ Solution I (bottle ❷).
- Drain surplus solution onto absorbent paper
- Dip the slide 2 x 1 second in RAL Diff-Quik™ Solution II (bottle ❸).
- Quickly rinse the slide with distilled water

**Histo-Cytology :****Length of one Test : 15 sec**

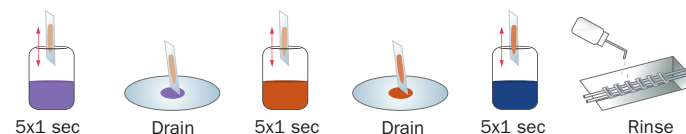
- Make a smear
- Dip the slide 5 seconds in RAL Diff-Quik Fixative (bottle ❶)
- Drain surplus solution onto absorbent paper
- Dip the slide 5 seconds in RAL Diff-Quik™ Solution I (bottle ❷).
- Drain surplus solution onto absorbent paper
- Dip the slide 5 seconds in RAL Diff-Quik™ Solution II (bottle ❸).
- Drain surplus solution onto absorbent paper

**Trichomonas****Length of one Test : 2 minutes and 15 sec**

- Dip the slide 15 seconds in RAL Diff-Quik Fixative (bottle ❶)
- Drain surplus solution onto absorbent paper
- Dip the slide 1 minute in RAL Diff-Quik™ Solution I (bottle ❷).
- Drain surplus solution onto absorbent paper
- Dip the slide 1 minute in RAL Diff-Quik™ Solution II (bottle ❸).
- Quickly rinse the slide with distilled water

**Histology : Detection of Helicobacter pylori**

- Dewax and hydrate the section
- Dip and shake the slide for 7 seconds in RAL Diff-Quik™ Solution I (bottle ❶).
- Dip and shake the slide for 5 seconds in RAL Diff-Quik™ Solution II (bottle ❷).
- Rinse with tap water and dry the slide onto filter paper
- Dip and shake the slide for 10 seconds in 90° Alcohol
- 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 :****Results:****Blood smear**

<b>Nuclei:</b>	Chromatin : ± dense purple
<b>Leukocytes:</b>	Cytoplasm without RNA : light pinkish Eosinophilic Granulations : orange-brown Basophilic Granulations : dark purple blue Neutrophilic Granulations : ± intense purple
<b>Lymphocytes:</b>	Cytoplasm with RNA : pure blue Cytoplasm without RNA : light blue Azurophilic Granulations : red
<b>Monocytes:</b>	Cytoplasm : grey-blue
<b>Erythrocytes:</b>	light red
<b>Platelets:</b>	Chromomere : purple-red Hyalomere : bluish
<b>Blood parasites:</b>	Nuclei : red
<b>(plasmodium)</b>	Cytoplasm : blue

**Parasitology and Mycology**

Cytoplasm 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**

<b>Helicobacter pylori:</b>	Dark Blue
<b>Nuclei:</b>	Blue
<b>Cytoplasm:</b>	Pink to red
<b>Collagen:</b>	Very pale pink

**Histo-Cyto**

<b>Nuclei:</b>	Red violet
<b>Cytoplasm:</b>	
<b>Acidophilic:</b>	Pink
<b>Basophilic:</b>	Blue
<b>Collagen:</b>	Pale pink
<b>Erythrocytes:</b>	Beige

RAL DIFF-QUIK™ FIXATIVE SOLUTION - 720560-0100  
-0500  
-2500



CONT	CH <sub>3</sub> OH
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DANGER. H225 Highly flammable liquid and vapour. H311 Toxic in contact with skin. H301 Toxic if swallowed. H331 Toxic if inhaled. 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



CONT	5-Chloro-2-methyl-2H-isothiazol-3-one/ 2-methyl-2H-isothiazol-3-one
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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

