

**Artisan  
Congo Red Stain Kit****Code AR161****Intended use**

For in vitro diagnostic use.

Congo Red Stain Kit is intended for histological staining to aid identification of amyloid in formalin-fixed, paraffin-embedded tissue section cut at 8 µm, by both standard and polarized light microscopy (1). The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls, and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist. All reagents in the Congo Red Stain Kit have been optimally prepared for use on Artisan Link and Artisan Link Pro Special Staining System and require no reconstitution or dilution.

**Summary and explanation**

The Congo Red Stain Kit is used to aid identification of amyloid in tissue sections. This procedure is based on the work of Puchtler and uses sodium chloride to reduce the background electrochemical staining and enhance the bond between Congo Red dye and amyloid (2).

**Principle of procedure**

The staining of amyloid by Congo red occurs when hydrogen bonds are established between the Congo red dye and the β-pleated sheet in a highly orientated linear and parallel manner on the amyloid fibrils (2-3).

The Congo Red Stain Kit is optimized for use on Artisan Link and Artisan Link Pro Special Staining Systems with or without Artisan Clearing Solution. Wash steps follow each of the staining steps. Following staining, the air-dried slides are removed from the instrument, cleared with xylene or xylene substitute and mounted with non-aqueous permanent mounting medium. Results are interpreted using both standard bright field microscope and using polarized light microscopy.

**Control Tissue**

Tissue with amyloid or heart tissue. **Note:** Tissue blocks and cut slides should be stored at 2-8 °C

**Reagents provided**

The Congo Red Stain Kit is composed of three ready-to-use dispenser packs, containing:

**Mayer's Hematoxylin****MAYER'S HEMATOXYLIN**

0.1% Hematoxylin, 5% aluminum ammonium sulfate and additives in deionized water

**Alkaline Alcohol****ALKALINE ALCOHOL**

80% Alcohol and sodium chloride

**Alcoholic Congo Red****ALCOHOLIC CONGO RED**

80% alcohol, 0.2% Congo Red and Sodium Chloride

**Materials required, but not supplied**

1. Microscope slides and coverslips
2. Artisan Link or Staining Link Pro Special Staining System
3. Polarizing filter
4. Artisan 50x Wash Solution (Code AR102)
5. Artisan Clearing Solution (Code AR309), optional
6. Mounting medium and ancillary reagents required for clearing and mounting
7. Control tissue

## Precautions

1. For in vitro diagnostic use.
2. For professional users.
3. Avoid microbial contamination of reagents or increase in nonspecific staining may occur.
4. Reagent packs may contain residual solution after completing the stated number of tests.
5. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any changes made to the factory-released procedure.
6. Store reagent packs in an upright position when not in use on the instrument.
7. Keep reagent packs out of direct sunlight and away from heat-generating sources.
8. As a general rule, persons under 18 years of age are not allowed to work with this product. Users must be carefully instructed in the proper work procedures, the dangerous properties of the product and the necessary safety instructions. Please refer to Safety Data Sheet (SDS) for additional information.
9. Wear appropriate Personal Protective Equipment to avoid contact with eyes and skin when opening hazardous material (hazmat) bag and during handling.
10. Unused solution should be disposed of according to local, State and Federal regulations.
11. Safety Data Sheet available for professional users on request.
12. For reagents shipped in sealed hazmat bags, check for any spilled liquid or broken reagent packs. If either of these conditions is present, do not open the hazmat bag. Return the bag and contents to the kit box and report damage to customer service for a replacement kit.



### Danger

**Alcoholic Congo Red:**  $\geq 50$  -  $\leq 75\%$  ethanol,  $\leq 4.7\%$  methanol,  $\leq 0.3\%$  disodium 3,3'-[[1,1'-biphenyl]-4,4'-diylbis(azo)]bis(4-aminonaphthalene-1-sulphonate)

H225	Highly flammable liquid and vapor.
H319	Causes serious eye irritation.
H350	May cause cancer.
H360	May damage fertility or the unborn child.
H371	May cause damage to organs. (central nervous system (CNS))
H335	May cause respiratory irritation.
H336	May cause drowsiness and dizziness.
H373	May cause damage to organs through prolonged or repeated exposure. (kidneys, liver)
P201	Obtain special instructions before use.
P202	Do not handle until all safety precautions have been read and understood.
P280	Wear protective gloves. Wear eye or face protection. Wear protective clothing.
P210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P241	Use explosion-proof electrical, ventilating, lighting and all material-handling equipment.
P242	Use only non-sparking tools.
P243	Take precautionary measures against static discharge.
P233	Keep container tightly closed.
P271	Use only outdoors or in a well-ventilated area.
P260	Do not breathe vapor.
P270	Do not eat, drink or smoke when using this product.
P264	Wash hands thoroughly after handling.
P314	Get medical attention if you feel unwell.
P308 + P311	IF exposed or concerned: Call a POISON CENTER or physician.
P304 + P340 + P312	IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER or physician if you feel unwell.
P303 + P361 + P353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water or shower.
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313	If eye irritation persists: Get medical attention.
P405	Store locked up.
P403	Store in a well-ventilated place.
P235	Keep cool.
P501	Dispose of contents and container in accordance with all local, regional, national and international regulations.



### Danger

**Alkaline Alcohol:**  $\geq 50$  -  $\leq 75\%$  ethanol,  $\leq 10\%$  propan-2-ol,  $\leq 4.7\%$  methanol

H225	Highly flammable liquid and vapor.
H319	Causes serious eye irritation.
H360	May damage fertility or the unborn child.
H371	May cause damage to organs. (central nervous system (CNS))
H335	May cause respiratory irritation.
H336	May cause drowsiness and dizziness.
H373	May cause damage to organs through prolonged or repeated exposure. (kidneys, liver)

P201	Obtain special instructions before use.
P202	Do not handle until all safety precautions have been read and understood.
P280	Wear protective gloves. Wear eye or face protection. Wear protective clothing.
P210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P241	Use explosion-proof electrical, ventilating, lighting and all material-handling equipment.
P242	Use only non-sparking tools.
P243	Take precautionary measures against static discharge.
P233	Keep container tightly closed.
P271	Use only outdoors or in a well-ventilated area.
P260	Do not breathe vapor.
P270	Do not eat, drink or smoke when using this product.
P264	Wash hands thoroughly after handling.
P314	Get medical attention if you feel unwell.
P308 + P311	IF exposed or concerned: Call a POISON CENTER or physician.
P304 + P340 + P312	IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER or physician if you feel unwell.
P303 + P361 + P353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water or shower.
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313	If eye irritation persists: Get medical attention.
P405	Store locked up.
P403	Store in a well-ventilated place.
P235	Keep cool.
P501	Dispose of contents and container in accordance with all local, regional, national and international regulations.



### Danger

**Mayer's Hematoxylin:** ≤10% chloral hydrate, ≤10% sulfuric acid, aluminum ammonium salt, hydrate (2:1:1:12)

H302	Harmful if swallowed.
H319	Causes serious eye irritation.
H315	Causes skin irritation.
H350	May cause cancer.
H373	May cause damage to organs through prolonged or repeated exposure. (kidneys)
P201	Obtain special instructions before use.
P202	Do not handle until all safety precautions have been read and understood.
P280	Wear protective gloves. Wear eye or face protection. Wear protective clothing.
P260	Do not breathe vapor.
P270	Do not eat, drink or smoke when using this product.
P264	Wash hands thoroughly after handling.
P314	Get medical attention if you feel unwell.
P308 + P313	IF exposed or concerned: Get medical attention.
P301 + P312 + P330	IF SWALLOWED: Call a POISON CENTER or physician if you feel unwell. Rinse mouth.
P302 + P352 + P362 + P364	IF ON SKIN: Wash with plenty of soap and water. Take off contaminated clothing and wash it before reuse.
P332 + P313	If skin irritation occurs: Get medical attention.
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313	If eye irritation persists: Get medical attention.
P405	Store locked up.
P501	Dispose of contents and container in accordance with all local, regional, national and international regulations.

### Storage

Store Alcoholic Congo Red, Alkaline Alcohol and Mayer's Hematoxylin at room temperature (15-30 °C). Do not use after the expiration date printed on the reagent pack. If reagents are stored under any conditions other than those specified, the conditions must be verified by the user. There are no obvious signs to indicate instability of this product. Therefore, appropriate controls should be run simultaneously using the same protocol as the patient specimens. If unexpected staining is observed which cannot be explained by variations in laboratory procedures or improper storage, and a problem with the reagents is suspected, contact Dako Technical Support.

### Instrument procedure / Staining procedure

#### Procedural notes

Please refer to the *Artisan Link Basic User Guide* for detailed instructions.

**The reagents are provided at optimal formulation for use according to the instructions and protocol provided for staining on prepared tissue sections. Any deviation from the recommended test procedures may give erroneous results.**

### Specimen preparation

Tissues preserved in neutral buffered formalin for routine processing and paraffin-embedding are suitable for use. The recommended section thickness is 8 µm. Deviation from the recommended thickness may affect staining performance.

**Priming reagent packs**

Prior to staining, reagent packs should be primed and checked for leakage. Check the prime of each reagent pack when a new kit is used and before the first staining run of the day.

1. Gently mix each reagent pack by inverting it 3-5 times.
2. If a red or black shipping cap is present on the dispense assembly of the reagent pack, remove the cap by gently pulling it down over a paper towel.
3. To prime a new reagent pack when it is first received, hold the reagent pack with the dispenser assembly pointed downward and lightly tap on the side of the reagent pack 2-3 times to release any air bubbles that may be trapped in the dispenser assembly.
4. Fully compress and release the plunger 3-4 times in rapid succession, taking care to absorb any expelled fluid into a paper towel, absorbent gauze, or similar material, until a steady stream is obtained.  
**NOTE:** be aware that some reagent packs contain hazardous chemicals, refer to the precautions section and the hazard pictograms printed on the reagent packs.
5. Before the first staining run of the day, reagent packs should be primed to ensure a steady stream by compressing and releasing the plunger 1-2 times.
6. Avoid dispensing excess amounts of reagent during priming. Excessive priming will result in reagent loss, which may cause the reagent pack to run out of fluid before the stated number of tests is performed.
7. Place the reagent pack on the Artisan Link or Artisan Link Pro Special Staining System by sliding the mounting guide on the back of the reagent pack into the slots on the reagent carousel until the pack is completely seated.

**Procedure**

Refer to the *Protocol* section in the DakoLink Software for the recommended detailed procedure. After staining is complete, slides will be dry. Dip slides in xylene or xylene substitute and mount using non-aqueous mounting medium.

**Staining interpretation**

*Bright field microscopy*

Amyloid, collagen, other fibrous materials: ..... **Pale pink/salmon**

Nuclei: ..... **Blue**

*Polarized light microscopy*

Amyloid: ..... **Apple green**










**Troubleshooting**

Refer to *Artisan Link Basic User Guide* or contact Dako Technical Support.

**References**

1. Carson FL, Capellano CH. Histotechnology a Self Instructional Text, 4<sup>th</sup> edition, American Society for Clinical Pathology Press, 2015.
2. Suvarna SK, Layton C, Bancroft JD. Theory and practice of histological techniques, 7<sup>th</sup> edition, pg. 271-280, 2013.
3. Glenner GG. Amyloid deposits and amyloidosis. The β-fibrilloses (in 2 parts), New England Journal of Medicine 302, 1283-1333, 1981.

**Explanation of symbols**

 REF	Catalogue number		Temperature limitation	 IVD	In vitro diagnostic medical device
	Manufacturer	 LOT	Batch code		Contains sufficient for <n> tests
	Use by		Consult instructions for use	 EC REP	Authorized representative in the European Community



Agilent Technologies, Inc.  
 5301 Stevens Creek Blvd.  
 Santa Clara, CA 95051  
 United States

Tel. +44 161 492 7050  
 www.agilent.com

TX02438/01

Revision 2020.07