STAINS

INSTRUCTIONS FOR USE

CE

DISCLAIMER

Aequip s.r.l. disclaims all liability with respect to damage to persons or property due to the improper use of this product and from failure to follow the directions, warnings, instructions and precautions set forth herein.

This “Instructions for Use” document should always accompany the device STAINS.

STAINS is manufactured by:

Aequip s.r.l.

Corso Castelfidardo 30/A, 10129 Turin, Italy

VAT number: 12251400011

SUMMARY

1. GENERAL INFORMATION

Commercial name	STAINS
Release	2.0.0
Variants	N.A.

The system is identified with the versioning of the following core modules:

ITEM	Version
Processing Core ITEM	2.0.0
Manager ITEM	2.0.0
Bucket ITEM	N.A. (simple storage)

STAINS is a stand-alone software IVD consisting of several modules compatible with the integration within third party solutions for image processing and visualization in anatomical pathology departments.

STAINS is composed by the following core modules with the following function:

1. **Manager ITEM**: receives the histological image to normalize excluding images that cannot be processed and sends the image to the Processing Core ITEM
2. **Processing Core ITEM**: provision of a normalized image thanks to an image processing algorithm for the normalization of stainings
3. **Bucket ITEM**: ITEM for the storage necessary for the processing.

This device is an *In vitro* diagnostic medical device and may coincide with the following designations in this user manual: medical device, device and/or IVD.

1.1. DEVICE CODE AND AUDIENCE

Commercial name	Basic UDI-DI	UDI-DI	UDI-PI	Ref. Code
STAINS	805612669STAINS2W7	08056126690016	(8012)2.0.0	STAINS

1.2. INTENDED PURPOSE

The device is used as part of a complex diagnostic activity, where the information received from the histological assessment is crucial and pivotal for the diagnostic decision. STAINS provides a more informative image, intended to aid diagnosis.

Specifically, STAINS modifies the chromatic profile of a histological image so that it is consistent with the reference image chromatic profile, without artifacts generation.

The device is intended to be used for the evaluation of the histological images stained with the histochemical Hematoxylin & Eosin (H&E) stain or stained with the immunohistochemical (IHC) stains. STAINS specifically works with IHC-stained images where the chromogen employed to stain biomarker-positive cell structures is

the 3,3′-Diaminobenzidine (DAB) that provides brown-colored staining and hematoxylin is employed as counterstain.

For the H&E stain, the device is intended to provide color normalization for the following organs:

1. Breast
2. Colon
3. Liver
4. Lung
5. Prostate
6. Stomach
7. Uterus

The normalization process for H&E-stained images consists in the modification of the chromatic profile of both stainings (Hematoxylin and Eosin).

For the IHC stains, the device is intended to provide color normalization for the following tissue and biological markers:

- Tissue: Breast
- Biomarkers: estrogen receptor (ER), human epidermal growth factor receptor 2 (HER2), Ki-67 marker (Ki67) and progesterone receptor (PgR).

The normalization process for IHC-stained images consists in the modification of the chromatic profile of Hematoxylin counterstain only, without changing the colors of DAB intensity-based structures.

The device autonomously detects the image quality and identifies the cases where the device should not complete the elaboration of the image.

The reference image, which will be defined during installation, will be chosen by the user in accordance with the pathologist's professional experience.

No contraindications are known or foreseen for STAINS use.

The device is intended for professional use only; thus, the intended users are professional pathologists.

1.3. RISK CLASS OF THE DEVICE

Classification according to Regulation (EU) 2017/746, Article 47, Annex VIII, **Rule 5a, Class A.**

Classification determined by:

«**Rule 5**
The following devices are classified as class A:

a) Products for general laboratory use, accessories which possess no critical characteristics, buffer solutions, washing solutions, and general culture media and histological stains, intended by the manufacturer to make them suitable for in vitro diagnostic procedures relating to a specific examination;
b) Instruments intended by the manufacturer specifically to be used for in vitro diagnostic procedures;
c) Specimen receptacles.»

STAINS is a stand-alone software IVD consisting of several modules compatible with the integration within third party solutions for image processing and visualization in anatomical pathology departments.

STAINS provides a more informative image, intended to aid diagnosis.

Specifically, STAINS modifies the chromatic profile of a histological image so that it is consistent with the reference image chromatic profile, without artifacts generation.

Thus, Rule 5a applies and the device is determined to be in Class A.

1.4. PRELIMINARY WARNINGS

Failure to observe the warnings below as well as the rules and precautions described in these Instructions for Use will immediately void any warranty on the device.

Manufacturer shall not be held responsible for any damage to persons or property as a result of failure to observe the standards or precautions listed below and set forth generally in this document.

	FAILURE OR NEGLECT TO COMPLY WITH THE FOLLOWING DIRECTIONS MAY RESULT IN THE INCORRECT USE OF THE DEVICE.
	DO NOT USE THE DEVICE UNTIL THESE INSTRUCTIONS FOR USE HAVE BEEN READ AND UNDERSTOOD IN THEIR ENTIRETY.
	NO MODIFICATION OF THE DEVICE AND/OR ITS PARTS IS ALLOWED.
	USING THE DEVICE FOR PURPOSES OTHER THAN THOSE INDICATED IN THESE INSTRUCTIONS FOR USE COULD INVALIDATE ITS PERFORMANCES.
	REPORT ANY SERIOUS INCIDENTS OCCURRING IN RELATION TO THE DEVICE TO THE MANUFACTURER AND THE COMPETENT AUTHORITY OF THE MEMBER STATE WHERE THE USER IS ESTABLISHED.
	IN THE EVENT OF A MALFUNCTION, REFER TO HOW TO HANDLE THE EVENT IN THE SPECIFIC SECTIONS RELATED TO THE USE OF THE PRODUCT.

1.5. PERFORMANCE

The normalization process of STAINS software was tested by computing the state-of-the-art full-reference image quality assessment metrics, in order to investigate the similarity measures between the original and the image normalized by STAINS. The image quality metrics prove the new release (**v2.0.0**) of STAINS device (in compliance with the Regulation (EU) 2017/746) does not introduce any significant variation in the final result with respect to the previously released CE-marked version (**v1.0.0**) of STAINS device (in compliance with the Directive 98/79/EC). More specifically, Peak signal-to-noise ratio (PSNR) values are always greater than 40 dB, threshold whereby, in digital pathology, no significant difference can be observed in the pathologists' evaluation. Pearson Correlation Coefficient (PCC) and Structural SIMilarity (SSIM) values result always greater than 99% for all H&E-stained tissues. In addition, the results of the quantitative performance analysis indicate that STAINS preserves the cellular structures and the information contained in the original image after the normalization process. A total of 14388 image fields from 7 H&E-stained tissues (breast, colon, liver, lung, prostate, stomach and uterus) and 2971 image fields from breast tissue stained with 4 IHC biomarkers (ER,

HER2, Ki67 and PgR) were processed for the validation phase. The average PCC and SSIM scores are always higher than 95% and 90% respectively, in H&E-stained images and greater than 99% and 97% respectively, in IHC-stained images; this proves the robustness of STAINS software in the quantitative performance test.

To evaluate the quality of normalization process performed by STAINS device, qualitative experiments were carried out involving end-users (i.e., the pathologists). Results of the qualitative testing analysis indicate that STAINS improves the image quality with a low impact artifact generation, especially in the worst-case scenario. The pathologists were invited to provide a clinical score on a 5-point qualitative scale (“1: bad”, “2: poor”, “3: acceptable”, “4: good,” and “5: excellent”) for the original image and the image processed by STAINS, after having selected one specific target image for each tissue/staining. For the 7 H&E-stained tissues (breast, colon, liver, lung, prostate, stomach and uterus) the average clinical scores are 4.810 and 3.714 for the normalized and original images, respectively. In addition, the percentage of cases where the clinical score of the normalized image is higher or equal than the original one, over the total number of cases, is 92.1%. For breast tissue IHC-stained with ER, HER2, Ki67 and PgR markers, the average clinical scores are 4.361 and 2.181 for the normalized and original images, respectively. In addition, the clinical score of the normalized image is always higher than the original one (100% of cases). This proves the improvement of image quality in the normalized images with respect to the original ones in clinical practice. The normalization process for IHC-stained images consists in the modification of the chromatic profile of hematoxylin counterstain only, without changing the colors of DAB intensity-based structures. This is evidenced by the result that none of the pathologists involved in the study observed diagnostically significant differences in color variation of DAB intensity-based structures between the original and normalized images.

1.6. ACCESSORIES AND DEVICES IN COMBINATION

The device does not require any accessory. Moreover, the device is not intended to be used in combination with any other device and/or product; however, the device is intended to be used on digitized images of samples which have previously been stained by specific stains.

1.7. MATERIALS

The device is a stand-alone SW; thus, it does not incorporate any material.

2. INSTALLATION

The device can be accessed through a cloud platform.

Aequip personnel are responsible for the initial setup and configuration. This includes generating a secure authorization token, activating the user profile and performing system checks to ensure proper functionality. Once the setup is complete, the user receives the authorization token, which can be used to interact with the software via RESTful API calls.

SW updates are implemented in the cloud platform as soon as Aequip releases them; thus, the latest software release is always available for the users.

On first installation, the user will be asked to select the target image for the specific tissue-stain pair. The user can provide his/her own target image or choose one from a batch provided by the manufacturer. The user must contact the manufacturer to change the target image.

	SW updates are intended by the manufacturer to achieve a variety of outcomes (enhanced performances, bug fixing, etc.) as well as to assure the safety of the device.

2.1. MINIMUM HW REQUIREMENTS

To ensure optimal interaction with the platform, it is recommended to use a device that meets the following minimum specifications:

- **RAM**: 8 GB
- **Internet connection**:
  - Download: 50 Mbps
  - Upload: 20 Mbps
- **Screen resolution**: Full HD 1080p 1920x1080 pixel

3. METHODS OF USE

	IT IS MANDATORY FOR THE SPECIALIST TO ALWAYS HAVE THE ORIGINAL IMAGE AVAILABLE, IN ORDER TO COMPARE IT WITH THE IMAGE NORMALIZED BY STAINS AND/OR VERIFY THE DIAGNOSIS USING THE ORIGINAL IMAGE.
	STAINS ALLOWS THE PROCESSING OF COMPLETELY ANONYMIZED ORIGINAL IMAGES THAT MUST NOT INCLUDE THE PATIENT IDENTIFICATION DATA.
	THE DEVICE IS NOT DESIGNED TO PROVIDE IMAGE PROCESSING IMMEDIATELY.
	THE INPUT (ORIGINAL IMAGE) AND OUTPUT (NORMALIZED IMAGE OR COLOR PROFILE) DATA ARE ARCHIVED FOR 3 DAYS ONLY. SUBSEQUENTLY, THE SOFTWARE WILL AUTOMATICALLY DELETE ALL DATA FROM STORAGE, AND THE LINK RELATED TO THE NORMALIZED IMAGE WILL NO LONGER BE VALID. THE VALUE RELATED TO DATA REMOVAL TIME IS SETTABLE IN A CONFIGURATION FILE. AUDIT LOGS OF PROCESSING ACTIONS PERFORMED ON IMAGES WILL BE ARCHIVED FOR TRACKING BY THE DEVELOPMENT TEAM.

3.1. MINIMUM WSI REQUIREMENTS

The Whole Slide Images (WSI) to be normalized with STAINS must satisfy the following requirements:

- **Magnification**: at least 10x and maximum up to 80x
- **Image format**:
  - .svs
  - .ndpi
  - .tif (.tiff)
  - .mrxs (.zip)

The maximum size allowed for uploading a file to the system is 4 GB. Files larger than this will not be accepted and processed by STAINS.

3.2. AUTHENTICATION

To use the device, you need an active license. Access is granted via token on cloud platform.

3.3. FUNCTIONING

The image to be normalized must be accompanied by the following information:

- **Staining**: H&E / IHC-ER / IHC-HER2 / IHC-Ki67 / IHC-PgR
- **Tissue**: Breast / Colon / Liver / Lung / Prostate / Stomach / Uterus
- **Image format**: .svs / .ndpi / .tif (.tiff) / .mrxs (.zip)
- **Direct link to data / file**
- **Output format (optional)**: image / profile
- **Processing priority (optional)**

Images in .mrxs format consist of a file with the .mrxs extension and a folder with the same name, containing the data that makes up the image itself. In order to process this type of format, it is necessary to insert a .zip folder containing the .mrxs file and the image data folder into the direct link.

The image to be processed is sent via API request to STAINS device; subsequently, the software performs the normalization process, modifying the chromatic spectrum of the image so as to make it consistent with that of the target image, avoiding the generation of artifacts. At the end of the process, the result of the normalization is made available to the user in a download link that, depending on output format information, can contain:

- Normalized image in .tif format
- Color profile in .cube format, to be applied to the original image to obtain the normalized image.

If the information in output format is “image”, STAINS will return the normalized image, if the information is “profile”, STAINS will return the color profile. If the information is not provided, STAINS will return the normalized image by default.

3.3.1. Sequence of actions to normalize an image

To interact with the API, it is necessary to use a client application such as Postman (https://www.postman.com/) or Insomnia (https://insomnia.rest/), or any other similar client the user is familiar with. These tools allow the user to send requests and view responses easily.

STEP 1

To perform the first step of image submission, two alternatives are available and described below (STEP 1a or STEP 1b):

a. Make a POST request to https://cert.cluster1.aequip.it/api/v0/job/ by inserting your token in the Authorization Header of the request (e.g. Token 0000000000000000000000000000000000000000). The body of the POST request must have the following characteristics:

i. **Type**: JSON
ii. **Content**: JSON in the following form

```
{
"img_url": "direct link to the image",
"metadata":{
            "filename": "filename" or "format": "image format",
            "stain": "stain",
            "tissue": "tissue"
            }
}
```

A “direct link to the image” is a URL that points straight to the image file, allowing it to be viewed or downloaded immediately without navigating through a webpage.
An optional tag “output_format” can be added to the “metadata”. This tag can take the value of “image” or “profile”. In the first case STAINS will return the normalized image (in .tif format), in the second it will return the color profile (in .cube format) to apply to the original image to obtain the normalization. If this tag is not provided, STAINS will return the normalized image by default.

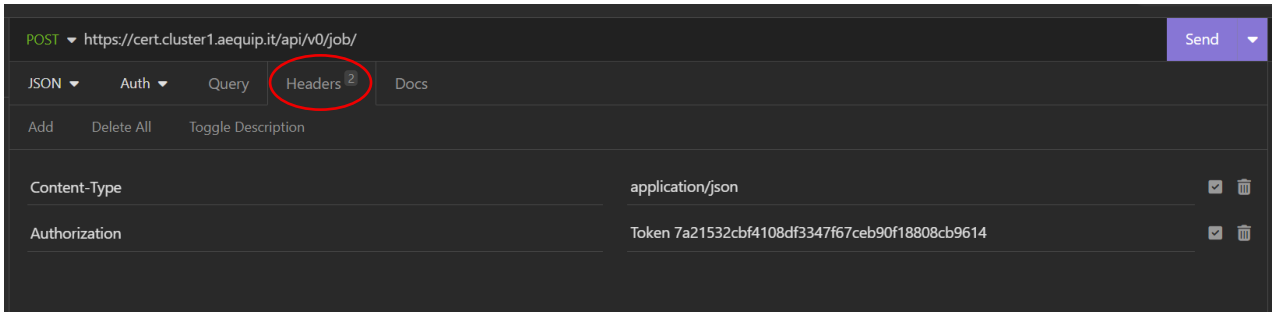

Figure 1. Example of a POST request (STEP 1a) using the Insomnia client. Headers to include when making a POST request.

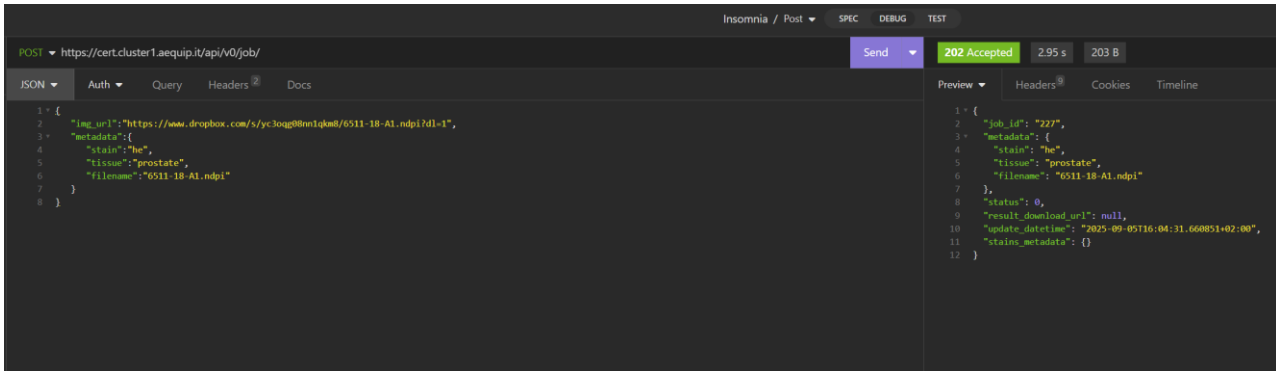

Figure 2. Example of a POST request (STEP 1a) using the Insomnia client. Body (json format) of the POST request. If the request is successful, a job_id will be assigned to the processing task and returned in the server's response.

b. Make a POST request to https://cert.cluster1.aequip.it/api/v0/job_fd by inserting your token in the Authorization Header of the request (e.g. Token 0000000000000000000000000000000000000000). The body of the POST request must have the following characteristics:

i. **Type**: Multipart
ii. **Content**:
    I. **Input**: file type field where the actual file is entered

II. **Metadata**: field containing metadata in the following form

```
{
"filename": "filename" or "format": "image format",
"stain": "stain",
"tissue": "tissue"
}
```

Also, in this case the optional tag “output_format” can be added in the “Metadata”.
For the POST request in STEP 1b, the tag “filename” or “format” in the metadata is optional.

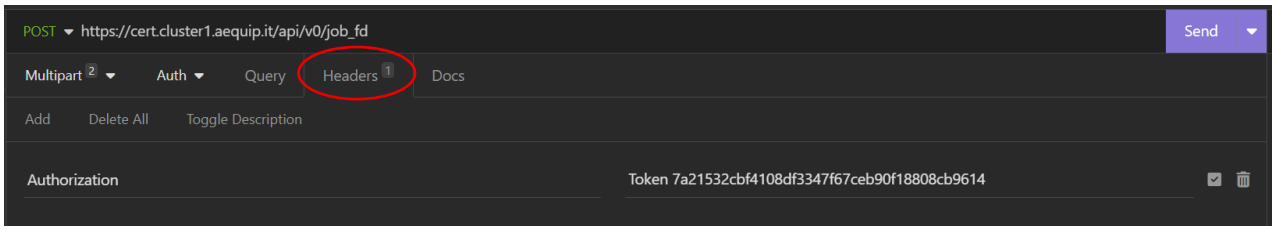

Figure 3. Example of a POST request (STEP 1b) using the Insomnia client. Headers to include when making a POST request. If the request is successful, a job_id will be assigned to the processing task and returned in the server's response.

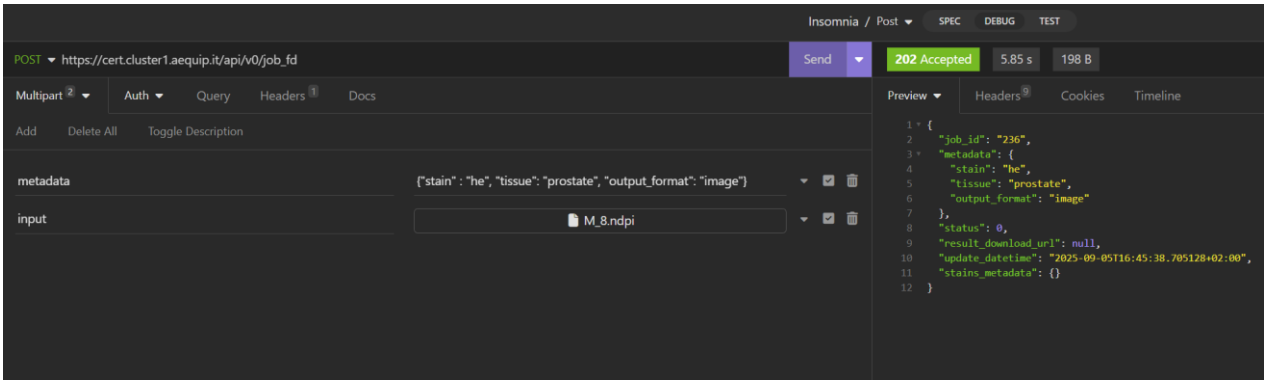

Figure 4. Example of a POST request (STEP 1b) using the Insomnia client. Body (Multipart format) of the POST request. If the request is successful, a job_id will be assigned to the processing task and returned in the server's response.

You can include processing priority information in the metadata of the POST request. This allows the system to prioritize the processing of an image over other non-priority jobs in the queue. To enable this feature, please contact the company.

STEP 2

Make a GET request to the address https://cert.cluster1.aequip.it/api/v0/job/job_id where “job_id” represents the unique number of the processing in progress, returned by the response to the POST request at STEP 1 under the heading “job_id”.

The response to the GET request will provide the processing progress status in the “status” heading. The “status” can have the following values:

-1	Request not accepted
0	Request accepted

1	Request in configuration phase
2	Request awaiting processing
3	Processing in progress
4	Processing completed successfully
5	Processing interrupted
6	Processing suspended
7	Processing failed
8	Processing cancelled
99999	Generic error

If the processing is successfully completed (“status”: 4), proceed to the next step. If an error occurs, repeat the sending as described in this section. If the error persists, contact support.

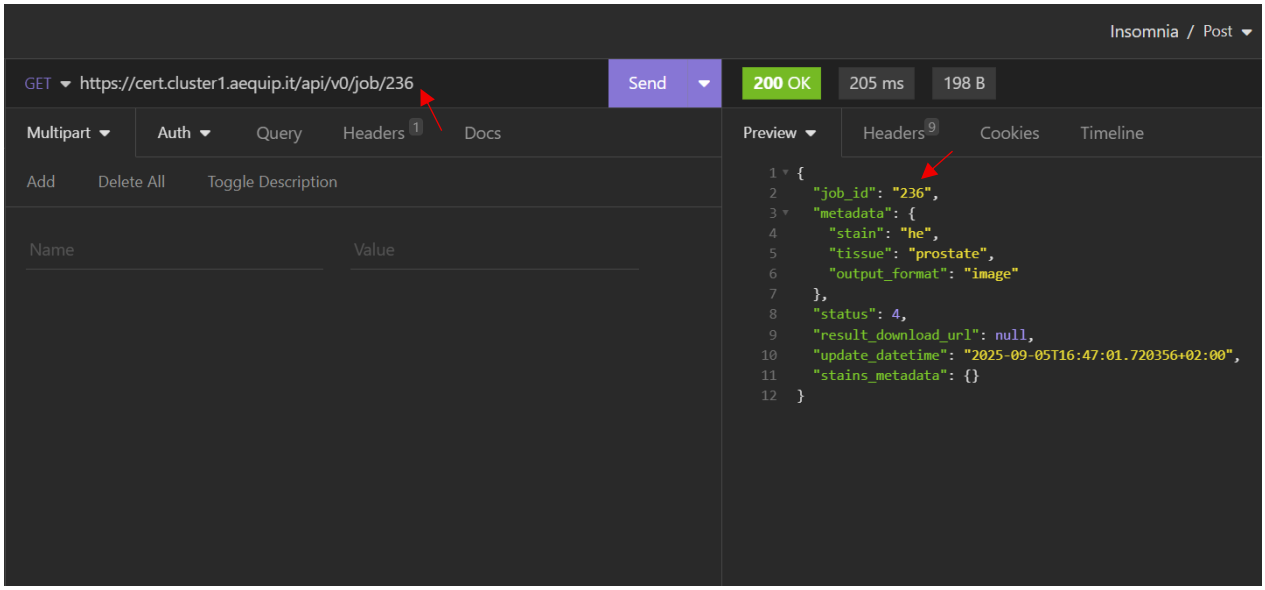

Figure 5. Example of a GET request (STEP 2) to check the processing status of a job identified by its unique job_id.

STEP 3

Once the processing is successfully completed, in order to obtain the processing result, make a GET request to https://cert.cluster1.aequip.it/api/v0/job/job_id/result. The response will provide the download URL of the output provided by STAINS under "result_download_url" if processing was successful.

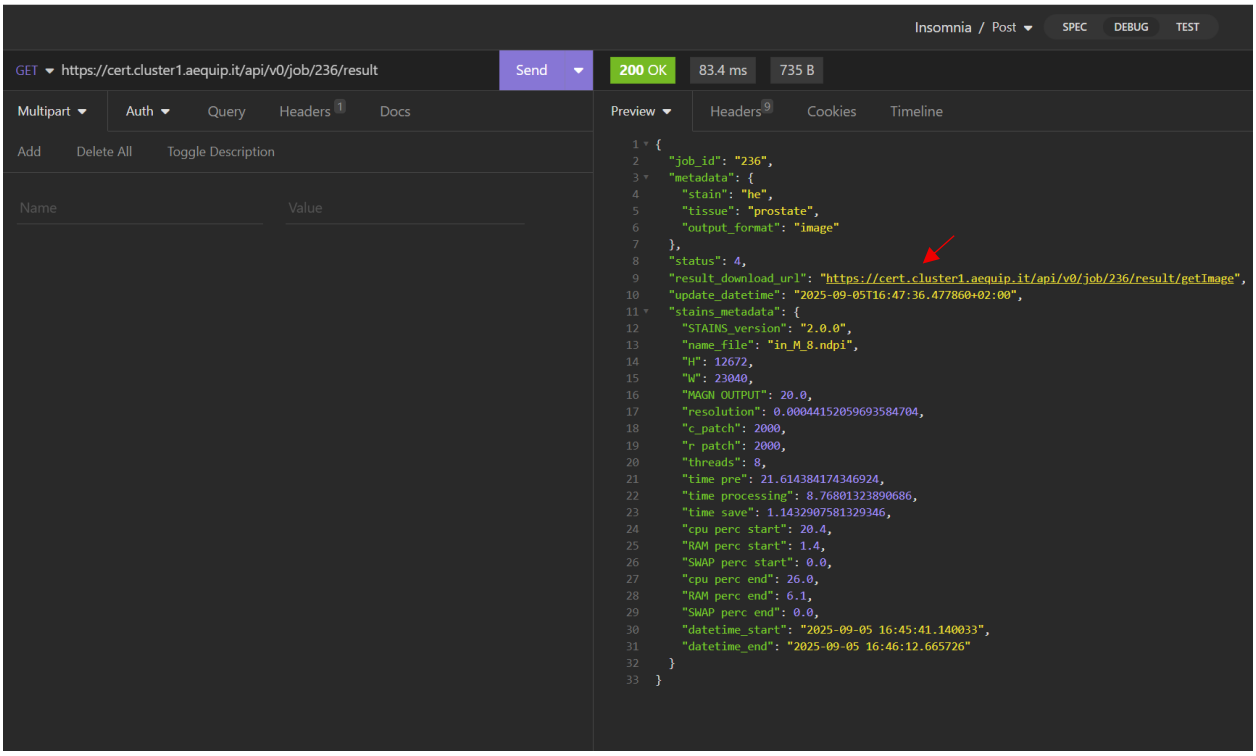

Figure 6. Example of a GET request (STEP 3) to retrieve the link of a successfully completed job. The response will include the download URL of the output provided by STAINS.

STEP 4

Make a GET request to https://cert.cluster1.aequip.it/api/v0/job/job_id/result/getImage, also indicated in the "result_download_url" entry of the response to STEP 3. This request will directly download the STAINS result.

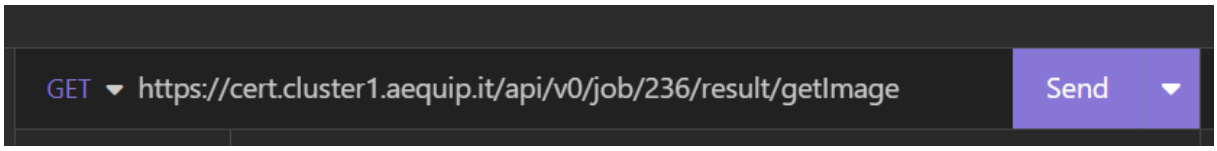

Figure 7. Example of a GET request (STEP 4) to download the output provided by STAINS.

3.3.2. Request for the UDI (Unique Device Identification) code

STAINS exposes an API that lets users retrieve the UDI (Unique Device Identification) code and the software version. To obtain this information, send a GET request to https://cert.cluster1.aequip.it/api/v0/udi and include a valid token in the Authorization header.

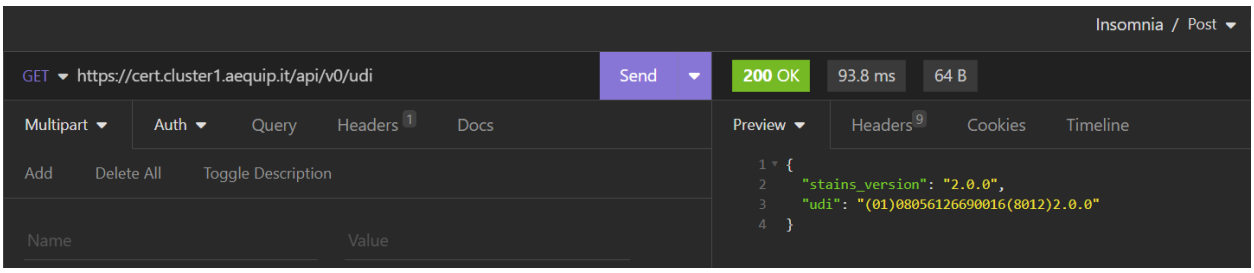

Figure 8. Example of a GET request to obtain the UDI (Unique Device Identification) code and the release version.

4. MAINTENANCE

4.1. SERVICE INTERRUPTION

To submit a service interruption request, contact customer service (refer to the "**Customer Service Contact Details**" section).

4.2. EXTRAORDINARY MAINTENANCE AND REPAIRS

In the event of a system failure, check that the image format, stain and tissue information have been correctly entered and that are compatible with the software requirements, as mentioned above.

If the information provided was not helpful, for further technical assistance, refer to the "**Customer Service Contact Details**" section.

4.2.1. Customer Service Contact Details

For technical assistance, contact the following mail address: info@aequip.it

5. LABELING

STAINS is a stand-alone SW without a user interface; thus, the label of the device is available in this IFU document, in the following section.

5.1. PRODUCT LABEL

ÆQUIP

Aequip s.r.l.
Address: Corso Castelfidardo 30/A,
10129 Turin, Italy
VAT number: 12251400011
Web: www.aequip.it
Mail: info@aequip.it

REF **STAINS**

IT **2025/09/10**

RELEASE: 2.0.0

https://www.aequip.it/wp-content/uploads/MANUALS2025/User_manual_stains.pdf

CE IVD

UDI (01)08056126690016(8012)2.0.0

6. SYMBOLOGY USED

	Manufacturer
IVD	In Vitro Diagnostic Medical Device
REF	Product identification code
IT	Country and date of production

YYYY/MM/DD	
	UDI code
 e-IFU indicator (link)	Consult the instructions for use. Electronic IFU available at the link.
	CE mark in compliance to Regulation (EU) 2017/746