



EU Reference Laboratory for ASF
Animal Health Research Centre
(CISA), INIA
Ctra Algete-El Casar s/n
28130, Valdeolmos, Spain



**European Union Reference
Laboratory for African Swine Fever
(ASF-EURL)**

Centro de Investigación en Sanidad Animal (CISA-INIA)

Ctra. de Algete a El Casar, s/n

28130 Valdeolmos (Madrid), Spain

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1. GENERAL INFORMATION

1.1. ORDERS FOR REAGENTS AND KITS

All orders must be sent to:

The EURL
eurl.asf@inia.csic.es

with copy to the Technical Direction
servict@inia.csic.es; vdt.cisa@inia.csic.es

ASF European Union Reference Laboratory, EURL-ASF
Centro de Investigación en Sanidad Animal (CISA/INIA-CSIC)
Ctra. de Algete a El Casar, s/n
28130 Valdeolmos (Madrid), Spain

The **conditions for order** are as follows:

It is necessary to identify all products using the catalogue description, specifying the quantity of each item required. For this item, you should send document of request: “*DESCRIPTION OF REQUEST*” or “*DESCRIPTION OF REQUEST (evaluation of ASF commercial diagnostic kits)*” including:

- The products to be supplied with the proposed specification.
- Full name and address of the client for delivery. Including telephone contact and e-mail.
- The billing address, with the fiscal identification number (VAT registration number). In case of countries without VAT number, please provide any other fiscal identity number in order to prepare the corresponding budget.
- Transport costs are not included in the catalogue prices, and if necessary will be added in each budget.
- Payment shall be made by the client immediately on receipt of the invoice.

For some services, the amount of standard reagents and reference material requested may be limited per NRL to assure provisions to other NRLs. Requirements of shipment permissions to several countries may entail changes in reagent presentations (i.e only lyophilization) and therefore several material could be not available.

Biological material being shared is subject to a **Biological Material Transfer Agreement (MTA)** between the Parties, with particular attention to the **1992 Convention on Biological Diversity (effective 29 December 1993)**.

Regarding the transport conditions for each service requested by NRLs, the cost is covered by the NRL requesting the diagnostic services and it would be dependent of market variations and budgeted at the time of order. Several services must be subject to a specific agreement between both Parties or to specific contracts through a bidding process governed by the procedures established by FAO.

- To proceed with the delivery:
 - As regards European Member States (MS), it is required to receive by e-mail the form “**acceptance biological material shipments from CISA**” signed by the client.
 - As regards of Non EU Members States Clients, the form “**acceptance biological material shipments from CISA**” must be accompanied with **all necessary import licences and approvals** or other consents (including import documents for products classified as live and infectious as may be required to enable the products to be imported into the client's country).

1.2. PAYMENT

The price payable by the client and described in the product list is the price prevailing at the date of despatch.

- I. The budget will be sent to the customer by e-mail. Once accepted (signed and stamped), it will be re-sent to us by email to the following address:

eurl.asf@inia.csic.es
with copy to
vdt.cisa@inia.csic.es; servict@inia.csic.es

Besides, we will need the document **acceptance biological material shipments from CISA** signed and stamped too, and for non EU countries the pertinent import permit.

- II. Once we receive the accepted budget and the acceptance form (and the import permit, if is necessary), we will send the reagents. Once the customer receive the reagent we will proceed with the invoice including detailed instructions for payment.

Payment should be made within 30 days of the invoice date. Please, include the invoice number as reference code in order to allow us to identify the income.

2. PRICE LIST: REAGENTS

Transport costs are not included in the catalogue prices, and will be added in each budget.

2.1. ASF REFERENCE REAGENTS

ASF REFERENCE REAGENTS FOR ASF-WOAH-INDIRECT ELISA						
Code	Product Description	Price (€)	Presentation		Volume/ Quantity	Determinations*
			Frozen	Lyophilised		
ASF-CP	ASF POSITIVE REFERENCE SERUM			√	1 ml	8.000
				√	0.5 ml	4.000
ASF-CL	ASF LIMIT REFERENCE SERUM			√	1 ml	8.000
				√	0.5 ml	4.000
ASF-CN	ASF NEGATIVE REFERENCE SERUM			√	0.5 ml	4.000
				√	1 ml	8.000
ASFV-Ag	ASFV SEMIPURIFIED ANTIGEN			√	0.5 ml	8.000

*The number of determinations is referred taking into consideration the working dilutions according are described in the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals WOA <https://www.woah.org/en/what-we-do/animal-health-and-welfare/animal-diseases/> and in the ASF-EURL web page [<http://asf-referencelab.info/asf/en/procedures-diagnosis/sops>]

ASF REFERENCE REAGENTS FOR ASF-WOAH-IMMUNOBLOTTING [IB]						
Code	Product Description	Price (€)	Presentation		Volume/ Quantity	Determinations*
			Frozen	Lyophilised		
ASF-CP	ASF POSITIVE REFERENCE SERUM			√	1 ml	50
				√	0.5 ml	25
ASF-CN	ASF NEGATIVE REFERENCE SERUM			√	1 ml	50
				√	0.5 ml	25
ASF-IB	ASF IMMUNOBLOTTING STRIPS				1 strip	1

*The number of determinations is referred taking into consideration the working dilutions according are described in the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals WOA <https://www.woah.org/en/what-we-do/animal-health-and-welfare/animal-diseases/> and in the ASF-EURL web page [<http://asf-referencelab.info/asf/en/procedures-diagnosis/sops>]

ASF REFERENCE REAGENTS FOR ASF WOA –INDIRECT IMMUNOPEROXIDASE TEST [IPT]					
Code	Product Description	Price (€)	Presentation		Volume/ Quantity
			Frozen	Lyophilised	
ASFV-IPT	COATED 96-WELL PLATES FIXED WITH ASFV ADAPTED ISOLATE (P72 GENOTYPE I)		√		1 Plate
ASF-CP	ASF POSITIVE REFERENCE SERUM			√	0.5 ml
ASF-CL	ASF LIMIT REFERENCE SERUM			√	0.5 ml
ASF-CN	ASF NEGATIVE REFERENCE SERUM			√	0.5 ml
ASFV-REF (E70MS-BA71VR)	ASFV ADAPTED VERO OR MS REFERENCE ISOLATE (P72 GENOTYPE I)		√		1 ml

ASF REFERENCE REAGENTS FOR ASF VIRUS/GENOME DETECTION

Code	Product Description	Price (€)	Presentation		Volume/ Quantity
			Frozen	Lyophilised	
ASF-ASFV ISOLATES	ASF REFERENCE VIRAL ISOLATES		√		1 ml
ASFV-DNAs	ASFV REFERENCE DNAs		√		100 µl

2.2. MATERIAL AND REAGENTS PROVIDED BY CISA-INIA FOR ASF DIAGNOSIS

CODE	ASF DIAGNOSTIC TEST	REAGENTS INCLUDED	DETERMINATIONS* (Nº)	PRICE (€)
ASF-WOAH-ELISA	WOAH-ELISA FOR ASF ANTIBODY DETECTION	<input type="checkbox"/> ASF-Ag 0.5 ml <input type="checkbox"/> ASF-CP 1 ml <input type="checkbox"/> ASF-CL 1 ml <input type="checkbox"/> ASF-CN 1 ml <input type="checkbox"/> HRP labelled Protein A 1 mg	8.000	
ASF-WOAH-IB	IMMUNOBLOTTING FOR ASF ANTIBODY DETECTION	<input type="checkbox"/> ASF-IB (60 strips) <input type="checkbox"/> ASF-CP 1 ml <input type="checkbox"/> ASF-CL 1 ml <input type="checkbox"/> ASF-CN 1 ml <input type="checkbox"/> HRP labelled Protein A 1 mg	50	
ASF-WOAH-IPT	INDIRECT IMMUNOPEROXIDASE TEST FOR ASF ANTIBODY DETECTION	<input type="checkbox"/> ASFV- IPT 3 plates <input type="checkbox"/> ASF-CP 25 □ <input type="checkbox"/> ASF-CL 25 □ <input type="checkbox"/> ASF-CN 25 □ <input type="checkbox"/> HRP labelled Protein A 1 mg	264	

*The number of determinations is referred taking into consideration the working dilutions according are described in the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (WOAH) <https://www.woah.org/en/what-we-do/animal-health-and-welfare/animal-diseases/> and in the ASF-EURL web page <http://asf-referencelab.info/asf/en/procedures-diaqnosis/sops>

2.3. REFERENCE MATERIAL FOR VALIDATION AND INTERNAL VERIFICATION OF ASF DIAGNOSTIC TECHNIQUES

ASF REFERENCE PANEL OF SAMPLES					
Code	Product Description*	Price (€)	Presentation		Volume
			Frozen	Lyophilised	
ASF-Ref-1	SERUM samples for the evaluation, validation and internal verification of ASF ANTIBODY detection techniques			√	1 ml/10 units
ASF-Ref-2	TISSUE AND SERUM samples for the evaluation, validation and internal verification of ASF-PCR techniques.			√	1 ml/16 units
ASF-Ref-3	ASFV DNAs for the evaluation, validation and internal verification of ASF-PCR techniques			√	100 □/21units

*All reference material has is **inactivated** by heat treatment. The description of the ASF-EURL reference material is available in the ASF-EURL web page.

3. AFRICAN SWINE FEVER: DIAGNOSTIC SERVICE

3.1. SUBMISSION OF SPECIMENS

To obtain the right diagnosis of a disease, it is essential to select the right samples and to send them to the laboratory under proper conditions. **It is not possible to make a good diagnosis if the samples are not in good conditions.**

3.1.1. Target samples for African swine fever

Sera: Draw whole blood collected using sterile tubes without anticoagulant. *Minimum amount recommended: 1 ml.*

Blood: Draw whole blood collected using sterile tubes with anticoagulant_(EDTA). *Minimum amount recommended: 1 ml.*

Porcine organs without formalin such as bone marrow, spleen, lung, kidney, heart, liver, tonsils, lymph nodes (mesenteric, retropharyngeal, renal, gastro-hepatic, etc). *Minimum amount recommended: 5gr.*

Organ samples should be obtained during the necropsy of the animal. Blood can be extracted via jugular vein puncture, the inferior vena cava, or the auricular veins (pigs), or during the necropsy.

3.1.2. Sampling materials required

- Watertight containers with hermetically sealable lids, preferably plastic flasks. These flasks should be used to keep the organs. Sterile tubes: some without anticoagulant others with EDTA. Some should be vacutainers and others not.
- Syringes of 10-20 ml. Different sizes of needles that fit the syringe and appropriate for the size of the pig to be sampled and the sampling site.
- Knife, scalpel and scissors to take the organ samples.
- Hermetically sealable flasks for storage of organ sample containers and blood tubes from each animal. These flasks should be clearly labeled.
- Cooler with ice packs or insulated box.
- Dry ice (when frozen sample is required).
- Labels and waterproof markers.

3.1.3 Shipping conditions

Samples should arrive at **CISA/INIA-CSIC as soon as possible** to avoid their deterioration and **safely** to avoid the possible infection of other animals or persons during transportation as well as to avoid contaminating the samples themselves. Ship specimens must be delivered with adequate amounts of ice packs to prevent deterioration. When delivery setup is delayed beyond 48 hours, samples should be **stored in liquid nitrogen or at -70°C**.

The specimens should be shipped in accordance with **IATA Packing Instruction 650 (UN3373) according is described below**.

The packaging must consist of three components:

- (a) a primary receptacle(s);
- (b) a secondary packaging; and
- (c) a rigid outer packaging.

Primary receptacles must be packed in secondary packaging in such a way that, under normal conditions of transport, they cannot break, be punctured or leak their contents into the secondary packaging. Secondary packaging must be secured in outer packaging with suitable cushioning material. Any leakage of the contents must not compromise the integrity of the cushioning material or of the outer packaging.

- Samples that must be shipped at 4 degrees Celsius with triple packaging: These samples should be packed with refrigerants (in sufficient quantity to maintain the desired temperature) within thermally insulated robust boxes. Preferably these boxes should be packed within other shock-resistant boxes. Rigid exterior packaging with cushioning material.
- Samples that must be shipped frozen (-20 degrees Celsius or -70 degrees Celsius): These materials should be packaged according adding enough dry ice to the thermally insulated box to maintain the temperature. It is important to ensure that the second container (described above) is secured within the center of the box because as the dry ice decomposes the container can become loose and damaged. The carbon dioxide resulting from the decomposition of the dry ice lowers the pH and deactivates the virus; therefore it is absolutely necessary that all the containers be hermetically sealed. Never freeze whole blood or serum that contains coagulant. Dry ice should never be placed in the secondary packaging. It should be placed between the secondary and the exterior packaging. The packaging must be designed to release carbon dioxide gas and prevent exposure.

3.1.4. Labelling

On the outside of the box the following identification should be placed:

1. Label reading 'biological material Category B'. UN 3373
2. Label reading 'infectious material for animals', if applicable.
3. Label reading 'dry ice' (if applicable) and net weight of dry ice.
4. Label reading 'conserve at 4 degrees Celsius' or 'conserve at -70 degrees Celsius'.
5. Full name and address and telephone number of sender.
6. Full name and address and telephone number of addressee.
7. Secondary risk label (risk label 9: miscellaneous when shipped with dry ice).

Dr. Carmina Gallardo

**Centro de Investigación en Sanidad Animal (CISA-INIA/CSIC)
ASF European Union Reference Laboratory, EURL-ASF**

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28130 Valdeolmos (Madrid), Spain
Tel: +34 91 620 23 00

SUBMISSION FORM MUST BE INCLUDED WITHIN THE PACKAGE OR SENT BY E-MAIL TO the EURL e-mail (eurl.asf@inia.csic.es) with copy to Dr. Carmina Gallardo (gallardo@inia.csic.es) and Dr. Raquel Nieto (nieto.raquel@inia.csic.es).

The **ASF submission form** is available on the website <http://asf-referencelab.info/asf/> or requesting by email to the ASF reference laboratory department (eurl.asf@inia.csic.es). If the general submission form is not available, the specimens must be accompanied by a covering letter. This letter should include relevant information concerning the owner, name and district of the farm, animal(s) involved, history, symptoms of illness and postmortem lesions. Test(s) required must be indicated and content list.

It is better to arrange the shipment with a “door to door” service courier that will ensure delivery directly to the Research Centre. The destination should be informed of the shipment by telephone or e-mail. It should have detailed information and approximate date and time of arrival. If material is sent by air, it should be given the flight number and the airway bill number.

3.2. ASF DIAGNOSTIC SERVICES

The laboratory offers an international diagnostic service for African swine fever, including the following tests:

ASF DIAGNOSIS	TARGET SAMPLES**	TEST DESCRIPTION*	AVERAGE TURNOVER TIME	PRICE(€)
ANTIBODY detection	<input type="checkbox"/> Serum samples (ELISA+ IPT)	<input type="checkbox"/> ELISA test (SOP/CISA/ASF/ELISA/2)	1 working day	/ per determination
	<input type="checkbox"/> Blood with EDTA and tissues (IPT)	<input type="checkbox"/> IPT (SOP/CISA/ASF/IPT/1)		
GENOME detection	Serum samples/ Blood with EDTA/ Tissues	<input type="checkbox"/> Real time PCR (SOP/CISA/ASF/PCR/3)	1 working day	/ per determination
VIRUS detection	Serum samples/ Blood with EDTA/ Tissues without formalin	<input type="checkbox"/> Virus isolation and identification by the haemadsorption test (HAD) in primary cultures (SOP/CISA/ASF/VI/1)	5 to 15 working days (1 to 3 passages)	/ per determination
ASFV GENOTYPING	Serum samples/ Blood with EDTA/ Tissues without formalin	<input type="checkbox"/> Sequencing using EURL standardized procedures (SOP/CISA/ASF/GENOTYPING/1)	5 working days	(total study including the corresponding report)

*Standard operating procedures (SOPs) are available in the ASF-EURL web page [<http://asf-referencelab.info/asf/en/procedures-diagnosis/sops>]

**See page 8 for more detailed information.

The free service for analysing samples from animals suspected to be infected with ASFV is available for up to 50 samples per country per year (except by prior agreement).

3.3. OTHER ASF RELATED SERVICES

SERVICE DESCRIPTION	PRICE(€)
Evaluation of ASF commercial diagnostic kits	STEP 1. Evaluation of ASF diagnostic kits using experimental samples/Assessment report
	STEP 2. Validation of ASF diagnostic kits using field samples/Validation report
	STEP 3. Verification of ASF diagnostic kits/Verification report
	TOTAL PRICE

4. TECHNICAL AND SCIENTIFIC ADVICE SERVICES: TRAINING EXPERTISE TO OTHER LABORATORIES

The laboratory offers **ASF scientific and technical individual short and long trainings courses at CISA-INIA** in order to transfer the ASF knowledge to Member States and third countries, with special emphasis to the ASF diagnostic tools available as URL for ASF. These courses aim to create a good coordination and cooperation for ASF disease control measures worldwide throughout ASF diagnostic harmonization.

The schedule of the training courses usually includes **hands-on exercises combined with theoretical lectures** focused on providing a general overview about the disease including history, epidemiology, control, and general aspects of the ASF diagnosis with special attention to the techniques to be performed during the laboratory practices activities. A set of practical information which includes copies of the lectures, laboratory practises results, Standard Operating Procedures (SOPs) and relevant ASF bibliography is provided to all participants in a digital format.

The cost to cover the ASF training courses at CISA-INIA includes:

- ⇒ **TECHNICAL AND SCIENTIFIC ADVISE** (€/hour):
- ⇒ **TECHNICAL AND SCIENTIFIC TRAINING** (€/per person-day):

SUBMISSION REQUEST FOR ASF TRAINING COURSES MUST BE SENT BY E-MAIL TO the EURL e-mail (eurl.asf@inia.csic.es) with copy to Dr. Carmina Gallardo (gallardo@inia.csic.es) and Dr. Marisa Arias (arias@inia.csic.es).