CatFlux Control and Test Solution

- Instructions for Use -

Restrictions on use

This product is intended exclusively for professional users in a medical diagnostic laboratory.

Intended purpose

The CatFlux Test and Control Solutions are intended to contribute to performing a CatSper Test, a laboratory test procedure based on a light microscopic assessment of sperm motility in a Ca^{2+}-free environment, to evaluate the functional status of CatSper Ca^{2+} channels of human sperm in semen samples provided by men seeking medical help for a suspected infertility with the purpose to identify an impaired CatSper function leading to CatSper-related male infertility (Young and Schiffer *et al.*, 2024), in a pseudo-quantitative, non-automated fashion, carried out by medical professionals (e.g., medical-technical assistants or comparable professional background) trained to examine and process human semen according to the standards set by the World Health Organization (WHO laboratory manual for the examination and processing of human semen, ISBN: 978 92 4 0030787), in a medical-diagnostic laboratory, separated from patients.

The CatSper Test is not to be used as the sole diagnostic criterion for determining the presence or absence of a pathological or physiological condition and does not serve as the basis for a treatment decision. The test result is only an indication of a possible dysfunction of the CatSper calcium channels of sperm and should always be interpreted by the physician in the context of other diagnostic tests and the patient's individual medical history.

Package contents and storage

One package contains ten sets of CatFlux Control and Test Solutions and one instructions for use.

Packages may be transported at ambient temperatures. Ensure the product is stored at 2 °C to 8° C upon receipt to maintain quality and efficacy for long-term storage.

Warnings and safety instructions

Only semen samples intended exclusively for diagnostic purposes, e.g., semen analysis, may be used. Semen samples that are intended for medical treatment, e.g., insemination, in vitro fertilization, or cryopreservation are not to be used in order to safely exclude any risk of unintentional contamination of the semen sample with the solutions. Use disposable pipettes or pipette tips and discard them after each pipetting step.

The CatFlux Test and Control Solutions are mixed with human semen during intended use. As a result, infectious agents such as HIV or Hepatitis C in the ejaculate can be introduced into the solutions, resulting in a potentially infectious mixture. Used CatFlux

Test and Control Solutions should, therefore, be treated as potentially infections material of human origin.

The CatFlux Control[1] and Test[2] Solutions contain bovine serum albumin (BSA)[1,2], NaCl[1,2], KCl[1,2], KH_2PO_4[1,2], $MgSO_4$[1], $CaCl_2$[1], HEPES[1,2], glucose[1,2], lactate[1,2], $NaHCO_3$[1,2], pyruvate[1,2], progesterone[2], EGTA[2], and EDTA[2] with trace amounts of DMSO[2]. Although the solutions are classified as non-hazardous mixtures according to Regulation (EU) No 1272/2008, CLP, the use of common laboratory personal protective equipment such as a laboratory coat, protective gloves, and eye protection should to be worn. Consult the material safety data sheet for safety-related instructions. Progesterone, while present in very low concentrations in the CatFlux Test Solution, may cause genetic defects, is suspected of causing cancer, and may damage fertility or the unborn child. Avoid direct contact with the CatFlux Test and Control Solutions.

In order to exclude the possibility of mixing up samples from different patients, it is recommended that the tubes are clearly and permanently labeled during use. In this way, test results can be reliably assigned to the correct patient.

Used CatFlux Test and Control Solutions are to be treated as potentially infectious laboratory waste and disposed of in accordance with all applicable national, local, and facility-specific regulations.

The CatFlux Solutions are only suitable and intended for performing a CatSperTest. The procedure described here was developed based on the methods described in the following scientific publication, which also serves as a reference for the scientific validity of the CatSperTest:

Young and Schiffer *et al.* (2024): Human fertilization in vivo and in vitro requires the CatSper channel to initiate sperm hyperactivation. *Journal of Clinical Investigation* https://doi.org/10.1172/JCI173564

The responsibility for performing a CatSper Test lies with the user.

Required materials

In addition to the CatFlux Test and Control Solutions, other materials and equipment are needed to perform the CatSper Test according to Young *et al.*, 2024. These include:

- Personal protective equipment. At a minimum, the wearing of a laboratory coat, protective gloves, and eye protection are strongly recommended. Consult the material safety data sheet for further instructions regarding safe handling, storage, and disposal of the product
- Refrigerator for storing the CatFlux Solutions before use (2 °C - 8 °C)
- Light microscope suitable for the examination of sperm motility according to the WHO manual
- Incubator (37 °C)
- Microscope slides and coverslips or comparable counting chamber
- Single-use micropipettes or micropipettes with disposable pipette tips – specifically for transferring a volume of 20 µl of ejaculate and the volume required to prepare a microscopic slide for determining sperm motility
- Laboratory waste containers according to regional specifications
- Stand for microreaction tubes

Specimen requirements

Semen samples should be collected in a sterile specimen receptacle according to the WHO manual. The CatSper Test is to be started within 30 to 60 minutes after sample collection. The start time is defined here as the time at which the semen sample is added to the CatFlux Test and Control Solutions (see “Performing the CatSper Test”).

Performing the CatSper Test

The steps for performing a CatSper Test according to Young *et al.*, 2024, for which the CatFlux Test and Control Solutions were designed, are described in the following:

1. Determine the fraction of motile sperm in the semen sample; follow the WHO recommendations for this. Samples with a fraction of motile sperm < 10 % cannot be reliably examined.

2. Retrieve a set of CatFlux Test and Control Solutions. Verify that they have not passed their expiration date. Inspect the solutions to ensure that they are clear to the naked eye (free of turbidity and/or debris, which might be possible signs of contamination). Please contact the manufacturer if you find signs of any type of contamination in the solutions that have not passed their expiration date.

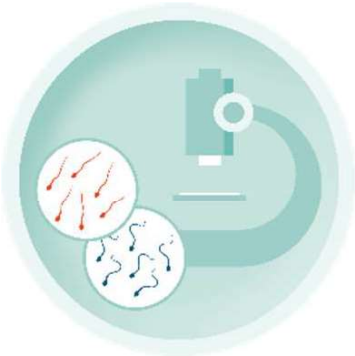
3. Warm the solutions by placing the tubes in a pre-warmed incubator (37 °C) for at least 5 minutes. Ensure that the CatFlux Solutions and the semen sample are at the same temperature before proceeding.

4. Pipette 20 µl of ejaculate from a fresh semen sample into the CatFlux Control Solution (blue tube) and CatFlux Test Solution (red tube).

5. Close the tubes securely and mix the contents carefully by repeatedly inverting the tube several times (15 seconds, although more viscous semen samples may require extra mixing). Finally, ensure that the bulk of the liquid mixture is resting at the bottom of the conical tube.

6. Incubate the two tubes for 60 minutes at 37 °C. The tubes should be placed in the incubator upright in a stand for microreaction tubes.

7. Remove the tubes from the incubator after 60 minutes. Once again, mix the contents of the tubes by inverting several times. Prepare a microscopic slide preparation of the sperm suspensions to determine the fraction of motile sperm as described in the WHO manual.



8. In both the Test and Control Solutions, record the motility of at least 200 sperm cells by categorizing them according to the WHO manual into motility classes A, B, C, and D.

9. Calculate the fraction of motile sperm in both solutions. For the CatSper Test, the fraction of motile sperm is defined by the sum of the progressively motile sperm (classes A + B) divided by the total number of sperm counted (Motility(A + B, %)).

10. Calculate the CatSper-Index (CI) according to the formula below. If the CI results in a negative number, it is to be evaluated as “0”. Document the test result.

$$CI = \frac{\text{Motility}(A + B, \%)_{\text{Control Solution}} - \text{Motility}(A + B, \%)_{\text{Test Solution}}}{\text{Motility}(A + B, \%)_{\text{Control Solution}}} \times 100$$

A CatSper Index result of less than 80 is an indication for a CatSper defect.

Examples of CatSper-Test results and calculated CatSper-Index values			
Example	Sperm motility (A + B, %)		CatSper Index
	Control Solution (blue)	Test Solution (red)	
1	55 %	0 %	(55 – 0)/55 x 100 = **100**
2	31 %	25 %	(31 – 25)/31 x 100 = **19**

Diagnostic sensitivity and specificity		
Sample	CatSper-intact semen sample	CatSper-defect semen sample
Number of samples (n)	61	6
Acceptance criteria (CatSper Index)	≥ 80	< 80
Positive	60	0
Negative	1	6
Sensitivity	100 %	
Specificity	98.3 %	
Accuracy	98.5 %	
Positive predictive value*	42 %	
Negative predictive value*	100 %	

*Based on a prevlance in the target group of 1.2 % (Young *et al.* 2024)

	Manufacturer		Date of manufacture		Use by date		Consult instructions for use	BIO	Contains biological material of animal origin		Contains hazardous substances
LOT	Batch code	REF	Catalog number		Keep away from sunlight	IVD	In vitro diagnostic medical device	Σ	Contains sufficient for 10 tests	UDI	Unique device identifier
	Keep dry		Temperature limit +2 to +8 °C		Do not re-use	CE	CE marking - manufactured in accordance with Regulation (EU) 2017/746 (IVDR) IVD class A				

We thank you for your trust!
Do you have any questions about our products?
Please feel free to contact us at
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