Elecsys PRO-C3

cobas®

Materials provided

REF		Σ	SYSTEM
09088423190	09088423500	100	**cobas** e 402 **cobas** e 801

For reagents, refer to the "Reagents" section.

Materials required (but not provided)

REF	Description
09088431190	CalSet PRO-C3, for 4 × 1.0 mL
09088440190	PreciControl PRO-C3, for 4 × 1.0 mL
	General laboratory equipment

Additional materials for **cobas** e 402 and **cobas** e 801 analyzers:

REF	Description
06908799190	ProCell II M, 2 × 2 L system solution
04880293190	CleanCell M, 2 × 2 L measuring cell cleaning solution
07485409001	Reservoir Cup, 8 cups to supply ProCell II M and CleanCell M
06908853190	PreClean II M, 2 × 2 L wash solution
05694302001	AssayTip/AssayCup tray, 6 magazines × 6 magazine stacks × 105 assay tips and 105 assay cups, 3 wasteliners
07485425001	Liquid Flow Cleaning Cup, 2 adaptor cups to supply ISE Cleaning Solution / Elecsys SysClean for Liquid Flow Cleaning Detection Unit
07485433001	PreWash Liquid Flow Cleaning Cup, 1 adaptor cup to supply ISE Cleaning Solution / Elecsys SysClean for Liquid Flow Cleaning PreWash Unit
11298500316	ISE Cleaning Solution / Elecsys SysClean, 5 × 100 mL system cleaning solution

Note

The measured N-terminal pro-peptides of type III collagen (PRO-C3) value of a patient's sample can vary depending on the testing procedure used. The laboratory finding must therefore always contain a statement on the PRO-C3 assay method used. Elecsys PRO-C3 values determined on patient samples cannot be used alone for clinical interpretation. **Values must be used as part of the ADAPT algorithm.** PRO-C3 values determined on patient samples by different testing procedures cannot be used for the same ADAPT algorithm and could be the cause of erroneous medical interpretations.

System information

Short name	ACN (application code number)
PRO-C3	10227

Intended use

Elecsys PRO-C3 is an in vitro quantitative immunoassay for the determination of N-terminal pro-peptide of type III collagen (PRO-C3) in human serum and plasma.

The assay is intended to be used as part of the ADAPT algorithm (patient age, diabetes status, PRO-C3, platelet count) to assess the severity of hepatic fibrosis in patients showing evidence or signs of metabolic dysfunction-associated steatotic liver disease (MASLD). The ADAPT result must be interpreted in conjunction with other methods and in accordance with standard clinical management guidelines.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on **cobas** e immunoassay analyzers.

Summary

The estimated global prevalence of metabolic dysfunction-associated steatotic liver disease (MASLD) in the general population has risen from 25 % in 2016 to currently more than 30 %, with the incidence continually increasing. The presence of MASLD is closely linked to type 2 diabetes (T2D), obesity and other cardiometabolic risk factors.[1] The diagnostic criteria for MASLD require the presence of hepatic steatosis along with at least one metabolic risk factor, such as obesity, type 2 diabetes, or components of metabolic syndrome. Metabolic dysfunction-associated steatohepatitis (MASH), a subset of MASLD, must involve evidence of hepatic steatosis, histological findings of steatohepatitis (such as hepatocyte ballooning, lobular inflammation, and/or fibrosis), and presence of at least one metabolic risk factor (such as obesity, type 2 diabetes, dyslipidemia, or hypertension).[1,2,3,4] Approximately 25 % of MASLD patients are estimated to have MASH, and 19 % of MASLD patients exhibit advanced fibrosis, typically presenting as asymptomatic but sometimes with fatigue, malaise, or right upper abdominal discomfort in MASH cases.[5,6,7] Age (> 50 years), insulin resistance, and multiple cardiometabolic risk factors all increase the probability of MASH.[1] Importantly, fibrosis stages F ≥ 3 are linked to higher risks of liver-related complications and mortality.[8]

The gold standard for the evaluation of liver fibrosis stage is percutaneous needle biopsy, which is compromised by inherent sampling and inter-observer biases and peri-procedural risk. The invasiveness and costs of performing biopsies also make it unsuitable for mass screening, staging, and risk stratification.[5,7] Non-invasive tools combine blood tests or integrate blood tests with imaging techniques that assess mechanical properties, hepatic fat content, and fibrogenesis.[9]

PRO-C3 is a marker of fibrogenesis, reflecting structural changes in the hepatic extracellular matrix (ECM) during chronic liver injury, and is valuable for assessing fibrosis progression or regression in liver disease.[5] It is derived from type III collagen, a fibrillar collagen composed of 3 α1(III)-chains forming a right-hand triple helix, secreted by fibroblasts and other mesenchymal cells. Type III collagen, along with type I collagen, constitutes a major part of the ECM and is secreted as a pro-peptide. During ECM incorporation, the N- and C-terminal propeptides are cleaved by specific proteases. The N-terminal propeptide of type III collagen (PRO-C3) is a specific marker of collagen formation, generated by the disease-specific N-protease, unlike the PIIINP epitope, which marks both formation and degradation.[9] Studies show a strong correlation between plasma PRO-C3 levels, severity of histological steatohepatitis, and advanced fibrosis (F ≥ 3).[3]

The ADAPT algorithm incorporates multiple clinical parameters: PRO-C3 levels, patient age, diabetes status, and platelet count. PRO-C3 is a biomarker that reflects the rate of type III collagen formation, offering direct insight into the active fibrogenic processes in the liver.[5] The European Association for the Study of the Liver (EASL) guidelines note that the PRO-C3 ADAPT test, which is based on components of collagen formation, provides additional evidence of fibrosis.[1]

Elevated PRO-C3 levels indicate ongoing fibrosis, making it a valuable marker for identifying patients with significant liver scarring. The ADAPT algorithm's integration of PRO-C3 provides cutoffs for fibrosis severity, guiding clinicians in identifying patients who require closer monitoring and more aggressive intervention. By leveraging the high specificity of PRO-C3 ADAPT, the algorithm reduces false positives, ensuring that patients who test positive for significant fibrosis are genuinely at risk.[5,7]

Note: The term metabolic dysfunction-associated steatotic liver disease (MASLD) has recently replaced the term "non-alcoholic fatty liver disease" (NAFLD) and similarly, metabolic dysfunction-associated steatohepatitis (MASH) replaced non-alcoholic steatohepatitis (NASH).[10]

Note: The biomarker for liver fibrosis used in the Elecsys PRO-C3 assay as a stand-alone marker does not provide the required performance for detecting significant fibrosis, advanced fibrosis, or cirrhosis to be used in clinical routine. All 4 ADAPT input values: platelet count, PRO-C3 assay result, diabetes status (yes/no), and patient age must be used.

Patient population

The Elecsys PRO-C3 assay with ADAPT should be used in an enriched adult (≥ 18 years) patient population, e.g. where patients have been referred for investigation due to abnormal biochemical tests (e.g., alanine aminotransferase [ALT], γ-glutamyltransferase [GGT] or FIB-4) or an ultrasonographically detected bright liver associated with features of metabolic syndrome. It should not be used in a primary care population without prior confirmatory tests. It is not intended for use in patients with other chronic liver disease including hepatitis B or C or patients with excessive alcohol consumption.

Test design and specificity considerations

The use of 90 % specificity cutoffs is designed to favor true negatives by ensuring that patients who test negative for the respective cutoffs are highly likely to not have the condition. This approach enhances diagnostic confidence and minimizes false positives. This approach can enhance the accuracy of identifying patients without the condition, as increasing specificity ensures fewer false positives, even though it may result in a reduction in sensitivity, meaning that a number of true positives might not be identified.

Algorithm score and fibrosis stages detection

The algorithm score indicates the severity of liver fibrosis and can be used to identify patients with significant fibrosis (fibrosis stage ≥ F2), advanced fibrosis (fibrosis stage ≥ F3), or cirrhosis (fibrosis stage F4), depending on the cutoff. Performance of detecting these different fibrosis stages varies by cutoff (see "Clinical performance of ADAPT for the detection of liver fibrosis at the respective cutoffs" section).

Test principle

Competition principle. Total duration of assay: 18 minutes.

- First incubation: 15 µL of sample, a biotinylated monoclonal PRO-C3-specific antibody, and a synthetic peptide PRO-C3 (145-153) labeled with a ruthenium complex[a)] react to form an immune complex. When the native PRO-C3 analyte exists in the sample, it competes with ruthenylated PRO-C3 synthetic peptide for the binding to the monoclonal PRO-C3-specific antibody.
- Second incubation: After streptavidin-coated microparticles have been added, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell, where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell II M. Application of a voltage to the electrode then induces chemiluminescent emission, which is measured by a photomultiplier.
- Results are determined via a calibration curve that is instrument-specifically generated by 2-point calibration and a leading calibration curve provided via **cobas** link.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Reagents

The **cobas** e pack is labeled as PRO-C3.

M	Streptavidin-coated microparticles, 1 bottle, 6.4 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.
R1	Anti-PRO-C3 Ab~biotin, 1 bottle, 8.6 mL: Biotinylated monoclonal anti-PRO-C3 antibody (mouse) 0.3 mg/L; phosphate buffer 40 mmol/L, pH 6.0; preservative.
R2	PRO-C3 (145-153)~Ru(bpy)$_3^{2+}$, 1 bottle, 8.2 mL: Synthetic peptide PRO-C3 (145-153) labeled with ruthenium complex 0.02 mg/L; phosphate buffer 40 mmol/L, pH 7.0; preservative.

Warnings and precautions

For in vitro diagnostic use for laboratory professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste

Warning: Handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards

Apply all relevant local disposal regulations to determine safe disposal.

The Safety Data Sheet is available for professional users on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H317	May cause an allergic skin reaction.

Prevention:

P261	Avoid breathing mist or vapours.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves.

Response:

P333 + P313	If skin irritation or rash occurs: Get medical advice/attention.
P362 + P364	Take off contaminated clothing and wash it before reuse.

Disposal:

P501	Dispose of contents/container to an approved waste disposal plant.

Hazardous components:

- 2-methyl-2H-isothiazol-3-one hydrochloride

Product safety labeling follows EU GHS guidance.

Contact phone for all countries: +49-621-7590

Avoid foam formation in all reagents and sample types (specimens, calibrators, and controls).

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the **cobas** e pack **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability	
unopened at 2-8 °C	up to the stated expiration date
on the analyzers	16 weeks

Calibration

Traceability: This method has been standardized against a purified reference material human PRO-C3 (145-153), which was quantified via amino acid analysis (AAA). The AAA was calibrated with a certified reference material (CRM) traceable to NIST SRM 84l and NIST SRM 350b.

The predefined leading calibration curve is adapted to the analyzer using the relevant calibrators.

Roller mix for at least 30 min after reconstitution. Ensure the calibrators are at 20-25 °C prior to measurement.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e., not more than 24 hours after the **cobas** e pack was registered on the analyzer).

The calibration interval may be extended based on acceptable calibration verification values determined by the laboratory.

Renewed calibration is recommended as follows:

- every 8 weeks when using the same reagent lot
- every 14 days when using the same **cobas** e pack on the analyzer
- as required, such as when quality control findings are outside the defined limits

Quality control

For routine quality control procedures, use PreciControl PRO-C3 or other suitable controls.

It is recommended to run the controls for the various concentration ranges individually at least once every 24 hours when the test is in use, once per **cobas** e pack, and following each calibration.

Roller mix for at least 30 min after reconstitution. Ensure the calibrators are at 20-25 °C prior to measurement.

Adjust the limits and control intervals based on the laboratory's individual requirements. If values fall outside the limits, each laboratory is advised to establish corrective measures.

If necessary, repeat sample measurement.

Follow the applicable government regulations and local guidelines.

Specimen collection and preparation

Serum collected using standard sampling tubes or tubes containing separator gel.

Only the specimens listed below were tested and found acceptable.

Li-heparin, K2 EDTA, and K3 EDTA plasma.

Li-heparin-plasma tubes containing separator gel can be used.

Criterion: slope 0.9-1.1, coefficient of correlation ≥ 0.90.

Stable for 2 days at 20-25 °C, 7 days at 2-8 °C, 12 weeks at -20 °C (± 5 °C).

The samples may be frozen up to 3 times.

(Acceptance criteria: For serum and plasma: ≤ 60 ng/mL ± 9.6 ng/mL; > 60 ng/mL ± 16 %.)

The sample types listed were tested with a selection of sample collection tubes or systems that were commercially available at the time of testing. Not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials that could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube / collection system manufacturer.

Centrifuge samples containing precipitates and thawed samples before performing the assay.

Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

Ensure the samples and calibrators are at 20-25 °C prior to measurement.

Due to possible evaporation effects, analyze and measure samples and calibrators on the analyzers within 2 hours.

Test procedure

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is available via **cobas** link.

For optimum performance of the assay, follow the instructions given in this document for the corresponding analyzer. For analyzer-specific assay instructions, refer to the corresponding User Guide.

Resuspension of the microparticles takes place automatically prior to use.

Place the cooled (stored at 2-8 °C) **cobas** e pack on the reagent manager.

Avoid foam formation.

The system automatically regulates the temperature of the reagents and the opening/closing of the reagent pack.

Calculation

The analyzer automatically calculates the analyte concentration of each sample in ng/mL.

Note: The value provided by the analyzer needs to be used in the ADAPT algorithm (see "Calculation of the ADAPT score and result interpretation" section).

If the PRO-C3 concentration of the samples is above 500 ng/mL, dilution of the sample is not necessary. The value to be used in the ADAPT algorithm for the PRO-C3 parameter is 500.

Limitations and interferences

The effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested. Interferences were tested up to the listed concentrations, and no impact on results was observed.

Endogenous substances

Compound	Concentration tested
Bilirubin	≤ 66 mg/dL or ≤ 1129 µmol/L
Hemoglobin	≤ 500 mg/dL or ≤ 0.310 mmol/L
Intralipid	≤ 2000 mg/dL
Biotin	≤ 1200 ng/mL
Rheumatoid factors	≤ 1200 IU/mL
IgG	≤ 7.0 g/dL
IgA	≤ 1.6 g/dL
IgM	≤ 1.0 g/dL
Albumin	≤ 7.0 g/dL

Criterion: Recovery ± 6.0 ng/mL of initial value ≤ 60 ng/mL and within ± 10 % of initial value > 60 ng/mL.

Commonly used pharmaceuticals

In vitro tests were performed on 17 commonly used pharmaceuticals. No interference with the assay was found.

In addition, the following special drugs were tested. No interference with the assay was found.

Special drugs

Drug	Concentration tested µg/mL
Ursodeoxycholic acid	8.409
Vitamin D	0.239
Semaglutide	0.083
Liraglutide	0.168
Fenofibrate	45
Niacin (nicotinic acid)	90
Empagliflozin	0.929
Losartan	0.672
Spironolactone	0.555

Drug interferences are measured based on recommendations given in the CLSI guidelines EP07 and EP37 and in other published literature. Effects of concentrations exceeding these recommendations have not been characterized.

In rare cases, interference may be caused by extremely high titers of antibodies to analyte-specific antibodies, streptavidin, or ruthenium. These effects are minimized by suitable test design.

For diagnostic purposes, always assess the results in conjunction with the patient's medical history, clinical examination, and other findings.

Limits and ranges

20-500 ng/mL (defined by the Limit of Quantitation and the maximum of the master curve). Values below the Limit of Quantitation are reported as < 20 ng/mL. Values above the measuring range are reported as > 500 ng/mL.

Lower limits of measurement

Limit of Blank, Limit of Detection, and Limit of Quantitation

Limit of Blank ≤ 3.0 ng/mL

Limit of Detection ≤ 6.0 ng/mL

Limit of Quantitation ≤ 20 ng/mL

The Limit of Blank, the Limit of Detection, and the Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th-percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low-concentration samples. The Limit of Detection corresponds to the lowest analyte concentration that can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is defined as the lowest amount of analyte in a sample that can be accurately quantitated with an intermediate precision CV of ≤ 20 %.

Linearity

The Elecsys PRO-C3 assay is linear across the measuring range from 20 to 500 ng/mL. Samples were prepared according to CLSI EP06-Ed2 by diluting 3 serum and 3 plasma sample sets each with low samples in multiple steps ranging from > 500 ng/mL downwards to the Limit of Quantitation.

Specific performance data

Representative performance data is given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, samples, and controls based on a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day, in duplicate each, for 21 days (n = 84). The following results were obtained:

cobas e 402 and cobas e 801 analyzers					
		Repeatability		Intermediate precision	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
Human serum 1	33.8	2.43	7.2	3.67	10.9
Human serum 2	44.8	2.11	4.7	3.34	7.4
Human serum 3	49.9	3.53	7.1	4.36	8.7
Human serum 4	60.5	2.66	4.4	4.21	7.0
Human serum 5	212	8.46	4.0	9.69	4.6
Human serum 6	296	10.2	3.5	12.7	4.3
Human serum 7	236	7.76	3.3	9.35	4.0
Human serum 8	390	16.9	4.3	17.0	4.4
PreciControl PRO-C3 1	37.1	1.54	4.1	2.74	7.4
PreciControl PRO-C3 2	239	8.06	3.4	9.99	4.2

Method comparison

A comparison of the Elecsys PRO-C3 assay, REF 09088423190 (**cobas** e 402 analyzer; y) with the Elecsys PRO-C3 assay, REF 09088423190 (**cobas** e 801 analyzer; x) gave the following correlations (ng/mL):

Number of serum samples measured: 103

Passing/Bablok[11]	Linear regression
y = 0.926x + 2.37	y = 0.816x + 10.1
τ = 0.904	r = 0.995

The sample concentrations were between 20.9 and 472 ng/mL.

Calculation of the ADAPT score and result interpretation

The ADAPT score can be manually calculated using the ADAPT algorithm (combining age, presence of diabetes, PRO-C3 concentration, and platelet count) provided below. The score is required to be calculated for each PRO-C3 result according to the intended use. The following steps serve as a guide for the manual workflow:

1. Order ADAPT and collect blood: 1 serum or plasma tube for PRO-C3 and 1 sample for the platelets count according to the respective laboratory requirements.

2. Send sample tubes plus information on age and the type 2 diabetes status (positive/negative) to the laboratory.

3. The laboratory receives the request and the sample tubes and tests for the PRO-C3 concentration and measures the platelets count.

4. The laboratory uses the provided formula to calculate the ADAPT score: The ADAPT algorithm is as follows:

$$ADAPT = exp\left(log_{10}\left(\frac{Age*ProC3}{\sqrt{Platelets}}\right)\right) + Diabetes$$

[ProC3] = ng/mL; [Platelets] = 10^9/L; [Diabetes] status positive = 1,
status negative = 0; [Age] = 18-100

- The ADAPT algorithm works with all positive results from the Elecsys PRO-C3 assay. Values below the LoQ will be set to LoQ; values above the HEoMR will be set to the high end of the measuring range. The following measurement limits have been used: LoQ 20 ng/mL and HEoMR: 500 ng/mL.
- The ADAPT algorithm accepts all plausible values in 10^9/L between 5 and 3500 as an input for platelet count.
- The ADAPT algorithm uses the information from diabetes status which has two categories labeled "positive" and "negative". For calculation, "positive" will be converted to 1 and "negative" will be converted to 0.
- The ADAPT algorithm accepts all plausible values in years as input for the age. Plausible values are defined from 18 to 100. Values > 100 and < 150 will be set to 100. Values > 150 and < 18 will be rejected.

5. Apply the 4-eyes principle when calculating the score.

6. The ADAPT score needs to be rounded to 3 digits.

7. The laboratory interprets the ADAPT score according to the "ADAPT score interpretation" section and enters all input values as well as the result (including the interpretation) in the laboratory information system. The examples below should be used to validate ADAPT calculations. The examples are simulated and not clinically relevant.

Elecsys PRO-C3 assay value (ng/mL)	Platelet count (10^9/L)	Age (years)	Diabetes status (positive = 1; negative = 0)	ADAPT score	ADAPT score interpretation
33.1	227.00	55	0	8.022	< 9.0: patient has no or mild fibrosis
27.9	67.51	55	0	9.692	≥ 9.0: patient has high risk for significant fibrosis (F2, F3 or F4)
94.1	599.43	45	1	10.374	≥ 10.0: patient has high risk for advanced fibrosis (F3 or F4)
91.9	67.49	70	1	19.062	≥ 11.0: patient has high risk of cirrhosis (F4)

ADAPT score interpretation

The ADAPT score received from the calculation has to be interpreted using the cutoffs in the following table:

ADAPT cutoff	**–**		**F2-F4 9.0**		**F3-F4 10.0**		**F4 11.0**	
ADAPT score	cutoff < 9.0		cutoff ≥ 9.0		cutoff ≥ 10.0		cutoff ≥ 11.0	
Fibrosis stage	F0, F1	not F2, F3, F4	F2, F3, F4	not F0, F1	F3, F4	not F0, F1, F2	F4	not F0, F1, F2, F3
Result interpretation	No or early/mild fibrosis		At least significant fibrosis		At least advanced fibrosis/pre-cirrhosis		Cirrhosis	
Result communication to physician	ADAPT score and cutoff Fibrosis severity of the patient Result interpretation ADAPT calculation input values: PRO-C3 (ng/mL), platelets count (platelets/µL blood), age (18-100), diabetes status (positive/negative)							

Example of result communication:

ADAPT cutoff	**ADAPT score**
≥ 10.0	**10.3**
Result interpretation • The calculated ADAPT score is evaluated with 3 predefined cutoffs (cutoff 1: 9.0; cutoff 2: 10.0; cutoff 3: 11.0).	

ADAPT cutoff	ADAPT score
≥ 10.0	10.3

- If the score is ≥ 9.0, the patient is at risk of having significant fibrosis (F2, F3, F4, but not F0, F1).
- If the score is ≥ 10.0, the patient is at risk of having advanced fibrosis (F3, F4, but not F0, F1, F2).
- If the score is ≥ 11.0, the patient is at risk of having cirrhosis (F4).
- The ADAPT result must be interpreted in conjunction with other methods and in accordance with standard clinical management guidelines.

Note: The cutoffs cannot be used in a combinatorial manner, i.e. patients cannot be identified for individual fibrosis stages.

Sensitivity and specificity

Clinical performance of the ADAPT score in a multicentric EU validation cohort (n = 683: F0 (84), F1 (140), F2 (207), F3 (179), and F4 (73)) at different cut-offs:

Cutoff	Sensitivity (95 % CI)	Specificity (95 % CI)
cutoff ≥ 9.0 Significant fibrosis (F2-F4)	52.7 % (48.2-57.2 %)	80.4 % (74.7-85.0 %)
cutoff ≥ 10.0 Advanced fibrosis (F3-F4)	49.6 % (43.5-55.7 %)	89.3 % (86.1-91.9 %)
cutoff ≥ 11.0 Cirrhosis (F4)	53.4 % (42.1-64.4 %)	90.0 % (87.4-92.1 %)

Result communication needs to contain all input values (PRO-C3 value (ng/mL), platelets count, diabetes status and age), the ADAPT score (with two decimals), the fibrosis risk assessment, as well as the result interpretation.

Clinical study for performance determination

A clinical study was conducted to assess the ability of the Elecsys PRO-C3 assay, combined with the ADAPT algorithm, to identify patients with significant fibrosis, advanced fibrosis, or cirrhosis. This prospective, EU multicenter trial included 683 patients showing evidence or signs of MASLD, with the following fibrosis stage distribution: F0 (84), F1 (140), F2 (207), F3 (179), and F4 (73).

Study cohort for the evaluation of ADAPT

The following table shows demographic characteristics (age, gender, diabetes status, and race distribution) for different fibrosis stages (F0-F4).

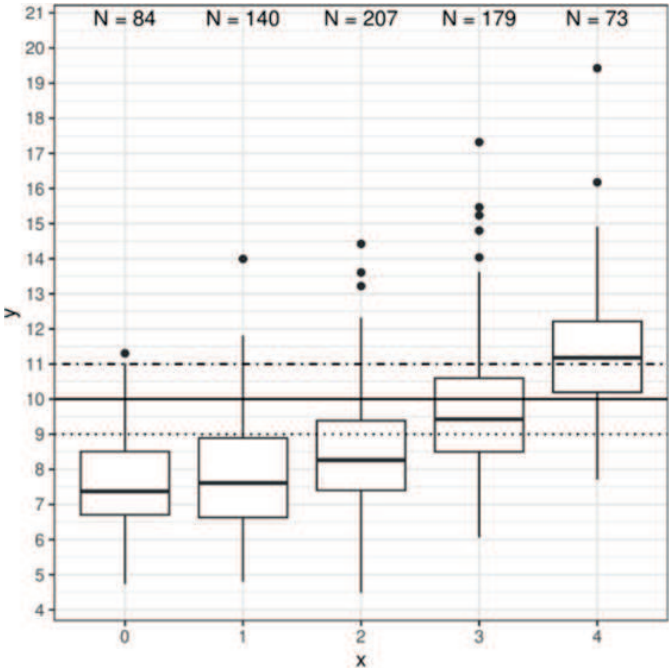
				Race				
	Median (age in years)	Gender (% male)	Diabetic (% true)	Asian (%)	Caucasian (%)	Black or African (%)	Other (%)	Missing (%)
F0	50.05	44	24	0	86	1	12	1
F1	47.12	54	36	3	87	1	8	1
F2	52.75	55	39	1	89	1	7	2
F3	59.56	49	69	4	87	0	8	1
F4	64.12	48	82	0	92	1	4	3

ADAPT score range in liver fibrosis stages

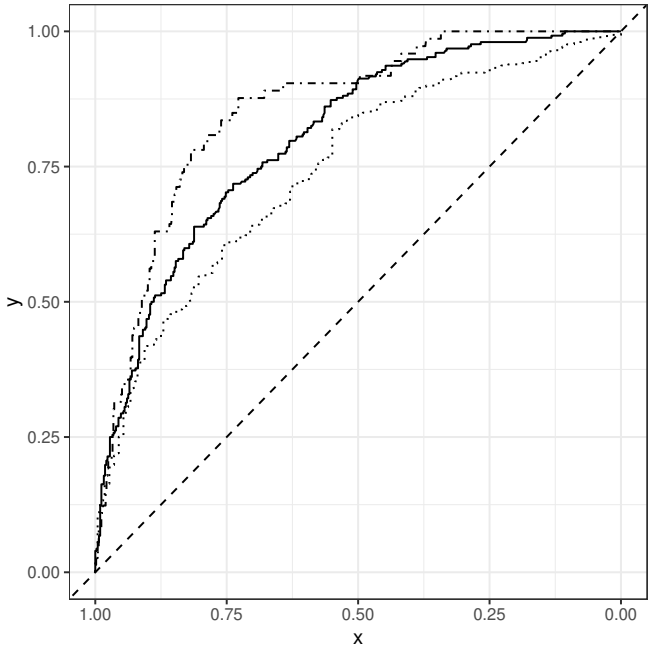
The following table shows the distribution of ADAPT scores across different fibrosis stages (F0 to F4), including the sample size (N), mean (SD), median, and interquartile range (25th-75th percentile) for each stage.

Fibrosis stage	N	Mean ADAPT score (SD)	Median ADAPT score	ADAPT score interquartile range
F0	84	7.55 (1.36)	7.38	6.69-8.53
F1	140	7.81 (1.66)	7.61	6.61-8.89
F2	207	8.39 (1.69)	8.27	7.39-9.43
F3	179	9.73	9.43	8.50-10.61

Fibrosis stage	N	Mean ADAPT score (SD)	Median ADAPT score	ADAPT score interquartile range
		(1.83)		
F4	73	11.29 (2.01)	11.18	10.20-12.22



ADAPT scores for individual fibrosis stages. Box plot representing the distribution of data: The box shows the interquartile range (IQR) with the median indicated by a line inside the box. Whiskers extend to 1.5 times the IQR, and points beyond the whiskers are considered outliers (solid circles). Horizontal lines represent ADAPT cutoffs for fibrosis stages: significant fibrosis (F2-F4) (dotted line), advanced fibrosis (F3-F4) (solid line), cirrhosis (F4) (dot-dashed line). x → fibrosis stage, y → ADAPT score.



Area under the curve (AUC) values (95 % CI) for receiver operator characteristic (ROC) curve to compare the diagnostic performance of ADAPT for significant fibrosis (F2-F4) 74.3 % (70.4-78.2 %) (dotted line) advanced fibrosis (F3-F4) 80.3 % (77.1-83.5 %) (solid line) and cirrhosis 85.4 % (81.3-89.4 %) (F4) (dot-dashed line). Long dashed line: line of no discrimination. x → specificity, y → sensitivity

AUC table ADAPT vs Elecsys PRO-C3 assay alone

The following table shows comparison of the Area under the curve (AUC) with 95 % confidence intervals (CI) for detecting significant fibrosis (F2-F4), advanced fibrosis (F3-F4), and cirrhosis (F4) between ADAPT and the PRO-C3 assay alone.

AUC (95 % CI)	Significant fibrosis (F2-F4)	Advanced fibrosis (F3-F4)	Cirrhosis (F4)
ADAPT	74.3 % (70.4-78.2 %)	80.3 % (77.1-83.5 %)	85.4 % (81.3-89.4 %)
Elecsys PRO-C3 assay alone	65.6 % (61.1-70.0 %)	65.8 % (61.5-70.2 %)	70.8 % (64.6-77.1 %)

Clinical performance of ADAPT for the detection of liver fibrosis at the respective cutoffs

ADAPT performance across fibrosis stages

The following table shows the diagnostic performance metrics for biopsy staging in 683 patients in detecting significant fibrosis (F2-F4), advanced fibrosis (F3-F4), and cirrhosis (F4), true positives, true negatives, false positives, false negatives, sensitivity, and specificity with 95 % confidence intervals (CI).

ADAPT diagnostic performance			
Performance metrics	**Significant fibrosis (F2-F4)**	**Advanced fibrosis (F3-F4)**	**Cirrhosis (F4)**
True positives	242	125	39
True negatives	180	385	549
False positives	44	46	61
False negatives	217	127	34
Sensitivity (95 % CI)	52.7 % (48.2-57.2 %)	49.6 % (43.5-55.7 %)	53.4 % (42.1-64.4 %)
Specificity (95 % CI)	80.4 % (74.7-85.0 %)	89.3 % (86.1-91.9 %)	90.0 % (87.4-92.1 %)

Significant fibrosis

ADAPT threshold	Sensitivity (95 % CI)	Specificity (95 % CI)
11	20.0 (16.6-23.9 %)	96.4 (93.1-98.2 %)
10	33.8 (29.6-38.2 %)	92.9 (88.7-95.6 %)
9	**52.7 (48.2-57.2 %)**	**80.4 (74.7-85.0 %)**
8	74.7 (70.6-78.5 %)	58.0 (51.5-64.3 %)
7	89.5 (86.4-92.0 %)	37.9 (31.8-44.5 %)

Advanced Fibrosis

ADAPT threshold	Sensitivity (95 % CI)	Specificity (95 % CI)
11	30.2 (24.8-36.1 %)	94.4 (91.8-96.2 %)
10	**49.6 (43.5-55.7 %)**	**89.3 (86.1-91.9 %)**
9	70.2 (64.3-75.5 %)	74.7 (70.4-78.6 %)
8	89.3 (84.9-92.5 %)	50.8 (46.1-55.5 %)
7	96.8 (93.9-98.4 %)	29.0 (24.9-33.5 %)

Cirrhosis

ADAPT threshold	Sensitivity (95 % CI)	Specificity (95 % CI)
11	**53.4 (42.1-64.4 %)**	**90.0 (87.4-92.1 %)**
10	78.1 (67.3-86.0 %)	81.3 (78.0-84.2 %)
9	90.4 (81.5-95.3 %)	63.9 (60.0-67.6 %
8	95.9 (88.6-98.6 %)	39.8 (36.0-43.8 %)
7	100 (95.0-100.0 %)	21.8 (18.7-25.3 %)

Additional information

For further information, refer to the User Guide for the corresponding analyzer, to the corresponding application sheets, and to the Method Sheets of all necessary components (if available in your country).

Additions, deletions, or changes are indicated by a change bar in the margin.

A point (period/stop) is always used in the English version of a Method Sheet as the decimal separator to mark the boundary between the integral and the fractional parts of a decimal numeral. The translated Method Sheets use decimal commas. Labels only use the decimal point as separator. Separators for thousands are not used.

Report any serious incident that has occurred in relation to the device to the manufacturer and the competent authority of the member state in which the user and/or patient is established.

The Summary of Safety & Performance can be found here:
https://ec.europa.eu/tools/eudamed

Symbols

In addition to the ISO 15223-1 standard, Roche Diagnostics uses the following symbols and signs:

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume for reconstitution
GTIN	Global Trade Item Number

References

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Changelog

For this document version only:

Due to technical reasons, changes that have been made since the last version of this document are listed in the following table instead of indicated by change bars in the margin.

Section headers are indicated in bold letters.

In addition to the changes listed in the table below, this method sheet version contains several editorial and layout updates.

Section	Current version	Previous version
Materials provided	**Materials provided**	**Order information** **Materials provided**
Materials required (but not provided)	**Materials required (but not provided)**	**Order information** **Materials required (but not provided)**
Note	**Note**	**Please note**
Reagents	**Reagents**	**Reagents - working solutions**
Warnings and precautions	**Warnings and precautions**	**Precautions and warnings**
Test procedure	**Test procedure**	**Reagent handling** **Assay**
Calculation of the ADAPT score and result interpretation	Table updated	
ADAPT score interpretation	Table updated	
ADAPT score range in liver fibrosis stages	Section updated	
ADAPT performance across fibrosis stages	Section updated	
Limitations and interferences	**Limitations and interferences**	**Limitations - interference**
Additional information	A point (period/stop) is always used in the English version of a Method Sheet as the decimal separator to mark the boundary between the integral and the fractional parts of a decimal numeral. The translated Method Sheets use decimal commas. Labels only use the decimal point as separator. Separators for thousands are not used.	A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.
Symbols	In addition to the ISO 15223-1 standard, Roche Diagnostics uses the following symbols and signs:	Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols):
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