PARACETAMOL
(Acetaminophen) Assay Kit

Instructions for Use

IVD — For *in vitro* diagnostic use only

Store in DARK at 2 to 8°C
DO NOT FREEZE

		K8001	K8002
REF			
REAG ENZ	Lyophilised Enzyme	1 vial	3 vials
DIL	Enzyme Diluent	1 x 45mL	1 x 45mL
REAG A	Colour Reagent A	1 x 65mL	1 x 65mL
REAG B	Colour Reagent B	1 x 65mL	1 x 65mL
CAL	2 mmol/L (302 mg/L) Aqueous Calibrator	1 x 3.0mL	2 x 3.0mL

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INTENDED USE
The Paracetamol (acetaminophen) assay is intended for the quantitative determination of paracetamol (acetaminophen) in human serum or plasma on clinical chemistry analysers or by manual spectrophotometric assays.

CLINICAL APPLICATION
Paracetamol (acetaminophen) is a commonly used analgesic which, if taken in excessive amounts, can lead to toxic liver damage and, less commonly, renal impairment.[1-3] The major metabolites of paracetamol are the glucuronide and sulphate derivatives. A small proportion of a metabolite formed by microsomal oxidation is conjugated to glutathione and excreted subsequently as cysteine or mercapturate conjugates. If the glutathione stores of the liver become depleted in the presence of a large amount of paracetamol, the oxidised metabolite combines with liver cell components causing hepatic necrosis.[4-5] The hepatocellular damage can be reduced by giving the patient compounds containing thiol groups such as methionine and N-acetyl cysteine.[6-12] The need to give one of these compounds is assessed on the measurement of the concentration of the parent drug in the blood, between four and twelve hours after ingestion.[7-8]

PRINCIPLE OF THE ASSAY
The method is based on the use of an enzyme specific for the amide bond of acylated aromatic amines. It cleaves the paracetamol molecule, yielding p-aminophenol, which reacts specifically with o-cresol in ammoniacal copper solution to produce a blue colour.[9,10,11] The assay is specific for the parent compound and does not detect paracetamol metabolites.

Paracetamol $\xrightarrow{\text{Aryl Acylamidase}}$ p-aminophenol + acetic acid

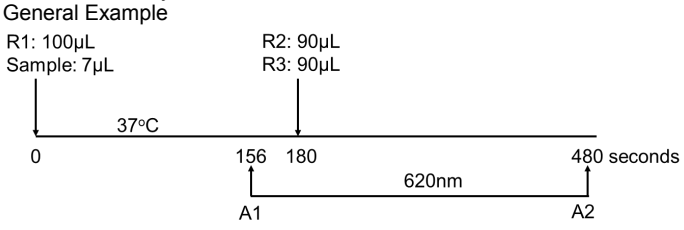
p-aminophenol + o-cresol + ammoniacal copper sulphate $\longrightarrow$ indophenol (blue)

SPECIMEN
Human serum or plasma are the recommended samples. For serum, ensure complete clot formation prior to centrifugation. For both serum and plasma, separate the red blood cells or gel as soon after collection as possible. Acceptable anticoagulants are heparin, EDTA, fluoride oxalate and citrate.

It should be noted that patient samples containing conjugates of paracetamol, which have been stored for more than two weeks at room temperature, may yield paracetamol as a result of degradation of the conjugates.

REAGENT PREPARATION AND STORAGE
Reconstitute each vial of lyophilised enzyme when required. Remove the cap and stopper from a bottle of lyophilised enzyme and add 10mL of enzyme diluent. Replace the stopper and swirl contents, inverting occasionally and ensure that the enzyme pellet is dissolved. Allow to stand at room temperature for five minutes. This becomes **Enzyme Reagent (R1)**. After reconstitution the enzyme reagent is stable for 4 months at 2 - 8°C or 1 month at 18 - 25°C or until the expiry date, whichever is sooner. **Colour Reagent A (R2)** and **Colour Reagent B (R3)** are ready to use.
If the lyophilised enzyme appears as a small hard pellet or is difficult to dissolve then the enzyme reagent should not be used.
Use the other reagents as supplied.

ASSAY PROCEDURE
The paracetamol calibrator (standard) is included with the reagents and should be used each time a new kit is started or a new vial of paracetamol enzyme is reconstituted. Re-calibration is recommended every 7 days.
Before each use, mix the reagents by gently inverting the vial. Avoid bubbles before placing on the clinical chemistry analysers.
For clinical chemistry analyser protocols, the settings are; endpoint, 2 point calibration with **CAL 1** (deionised H_2O or saline) set to **0mmol/L** (0mg/L) and **CAL 2** set to **2mmol/L** (302mg/L). Use water or saline as the zero calibrator.
Protocols are available for most clinical chemistry analysers. This can also be run as 2 reagents for clinical chemistry analysers that only have 2 reagent positions. Please contact Customer Services at Cambridge Life Sciences or your local distributor for further information.
General Example



THERAPEUTIC RANGE
Therapeutic concentrations vary significantly depending on the individual patient. A range of 66-199µmol/L (10-30mg/L) may be an effective sample concentration in many patients[13].
Toxic concentrations are >1.99mmol/L (300mg/L) at four hours after ingestion and >0.33mmol/L (50mg/L) after 12 hours. For diagnostic purposes, the test findings should always be assessed in conjunction with the patient's medical history, clinical examinations and other findings.

RESULTS
Paracetamol (acetaminophen) concentration is reported as mmol/L.
To convert results to mg/L, use the following conversion factor:
mmol/L x 151 = mg/L; mg/L ÷ 151 = mmol/L.
The paracetamol (acetaminophen) value should be used in conjunction with information available clinical evaluations and other diagnostic procedures.

QUALITY CONTROL
Good laboratory practice requires that quality control specimens be included in every run to monitor assay performance. The quality control samples should be assayed repeatedly to establish mean values and working ranges. A minimum of two levels of controls spanning the medical decision range is recommended to be run daily. If quality control results do not meet the acceptance criteria then recalibration may be necessary.

PERFORMANCE
Data presented using CLS Paracetamol (acetaminophen) reagent were preformed on an automated clinical chemistry analyser using an endpoint test mode.

Reportable Range
The reportable range is dependent on the sample to reagent ratio. The linearity is 0.02 - 3.00mmol/L (3 - 454mg/L). The regression equation against the target value is:
$y = 1.0014x + 0.32$ (mg/L), $r^2 = 0.9999$

Limit of Detection
A drug-free sera sample was tested in 20 replicates and the mean + 3SD = 0.012mmol/L (1.75mg/L). LoD = 0.02mmol/L (3.0mg/L).

Recovery (Linearity)
Seven levels of paracetamol linearity material were run. The mean for each sample was determined and the % recovery calculated. Representative results are shown.

Target (mmol/L)	Mean (mmol/L)	Difference (mmol/L)	Target (mg/L)	Mean (mg/L)	Difference (mg/L)	Recovery %
0.31	**0.320**	0.010	**46.87**	**48.36**	1.49	**103.2**
0.62	**0.626**	0.006	**93.74**	**94.49**	0.75	**100.8**
1.26	**1.235**	-0.025	**190.51**	**186.51**	-4.01	**97.9**
2.52	**2.541**	0.021	**381.02**	**383.74**	2.72	**100.7**
3.00	**3.011**	0.011	**453.6**	**454.71**	1.11	**100.2**

Precision
Typical precision for the assay is as follows:
Intra assay precision was for twenty determinations and Inter assay precision was over 15 days.

		Conc (mmol/L)	Std Dev. (mmol/L)	Conc (mg/L)	Std. Dev. (mg/L)	%CV
Intra Assay	**Sample 1**	0.145	0.0050	21.93	0.748	3.4
	Sample 2	0.260	0.0045	39.31	0.677	1.7
	Sample 3	0.736	0.0066	111.18	0.992	0.9
Inter Assay	**Sample 1**	0.054	0.0028	8.14	0.42	5.1
	Sample 2	0.243	0.0044	36.67	0.67	1.8
	Sample 3	0.700	0.0069	105.78	1.04	1.0

Accuracy
When patient serum samples were assayed and the results compared with those obtained by using the K8003 kit, the following regression equation resulted: where y = K8002 and x = K8003

$y = 0.995x - 0.36$ (mg/L), $r^2 = 0.9998$, n = 47

Correlation to an external quality scheme gave the following regression equation:

$y = 1.003x - 3.03$ (mg/L), $r^2 = 0.9998$, n = 23

Interferences
No interference was found using heparin, EDTA, fluoride oxalate and citrate blood collection tubes.

The common interfering endogenous substances of ascorbic acid, total bilirubin (unconjugated), direct bilirubin (conjugated), haemoglobin, triglycerides and intralipids show no significant interference up to the concentrations summarised in the table below.
Lipids may be removed using Lipoclear tubes taking into account the 20% dilution effect.

Interferent	Concentration
Ascorbic Acid	1.76 g/L
Total Bilirubin (unconjugated)	300.0 mg/L
Direct Bilirubin (conjugated)	300.0 mg/L
Haemoglobin	5.0 g/L
Triglycerides	10.0 g/L

The method does not measure the common metabolites of paracetamol (glucuronide, sulphate, cysteine and mercapturate). In addition, no reaction was obtained with the following drugs at a concentration of 1 mmol/L:

n-acetyl cysteine‡
acetylsalicylic acid†
amylobarbitone
amitryptyline
amphetamine
caffeine
chlordiazepoxide*
chlormezanone
chlorpropamide
dextropropoxyphene
diazepam*
dihydrocodeine
diphenydramine
impiramine
indomethacin
lorazepam*
meprobamate
methadone
methaqualone
nitrazepam*
oxypertine
p-aminosalicylic acid
pentazocine
p-ethoxyacetanilide (phenacetin)
p-ethoxyaniline (phenetidin)
phenobarbitone
phenytoin
promethazine
salicylamide
salicylic acid†
salicyluric acid
secobarbitone
sodium barbitone
theophylline
tolbutamide

* Tested at recorded peak plasma overdose concentrations (less than 1 mmol/L).
† Tested at a concentration of 5 mmol/L.
‡ Tested at a concentration of 1 g/L.

Important: Although method does not indicate any significant interference (under recovery) in the presence of n-acetyl cysteine, users of this product with automated analysers should follow the Cambridge Life Sciences protocols to avoid any possible interference. Any changes to these protocols should be verified by the user.

CALIBRATOR STANDARDISATION
Paracetamol calibrators are manufactured using primary calibration material, Acetaminophen (98.0% - 101.0%) that meets USP specifications. They are manufactured gravimetrically and tested against independent controls.

WARNINGS AND PRECAUTIONS
For in-vitro diagnostic use only.
For Professional use only.


Colour Reagent A contains 0.9% o-cresol.

Signal Word: Danger
Hazard Statements:
H301 - Toxic if swallowed
H311 - Toxic in contact with skin
H314 - Causes severe skin burns and eye damage
Precautionary Statements:
P280 - Wear protective gloves/ eye protection/ face protection.
P301 + P310 - IF SWALLOWED: Immediately call a POISON CENTRE or doctor/physician.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310 – Immediately call a POISON CENTRE or doctor/physician.

Colour Reagent B is irritating to eyes. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
Used samples, controls and pipette tips should be handled as clinical waste and incinerated or disposed of in accordance with local rules. Other reagents should be diluted and flushed down the drain. It is recommended that gloves be worn when handling such items.

Safety data sheets are available upon request from your local representative.

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