BECKMAN COULTER

CE 2797

Thymidine Kinase REA KIT

Instruction for use in local language is available at beckmancoulter.com/techdocs.

REVISION HISTORY

Previous version: PI-IM1948-05	**Current version:** IFU-IM1948-01
—	IVDR requirements incorporated
Chapter INTENDED USE removed	Chapter INTENDED PURPOSE added
—	Chapter APPENDIX: Interference data added

REF IM1948

FOR PROFESSIONAL USE ONLY

INTENDED PURPOSE

Thymidine Kinase REA KIT is an in vitro diagnostic manual medical device intended to be used by healthcare professionals for the quantitative measurement of thymidine kinase enzymatic activity in human serum and plasma. Measurement of thymidine kinase is intended to be used in the primary diagnosis and follow-up of patients with malignancies of the hematopoietic system and in the combination as a complementary tumour marker to the tumour cell proliferation assessment [1, 2, 3].

PRINCIPLE

There are two thymidine kinase (TK) isoenzymes in eucaryotic cells, TK 1 and TK 2, differing biochemically and electrophoretically. This radioenzymatic assay has been optimised for TK 1 estimation (also known as fetal TK, dTK-F, or cytosol-TK).

The radioenzymatic assay of thymidine kinase is based on enzymatic phosphorylation of 5-[^{125}I]-deoxyuridine (^{125}I-Tracer), catalysed by thymidine kinase contained in the sample or calibrator. 5-[^{125}I]-deoxyuridine is transformed to 5-iododeoxyuridine monophosphate (^{125}Id-UMP). ^{125}Id-UMP is then separated from the reaction mixture by adsorption on ion exchange resin. The sorbent is rinsed to remove 5-[^{125}I]-deoxyuridine. The radioactivity is then determined in a gamma counter. The TK enzymatic activity in the samples is obtained by interpolation from the standard curve. The enzymatic activity is directly proportional to the radioactivity.

WARNING AND PRECAUTIONS

General remarks:

- The vials with calibrators and controls should be opened as shortly as possible to avoid excessive evaporation.
- Do not mix the reagents from kits of different lots.
- A standard curve must be established with each assay.
- It is recommended to perform the assay in duplicate.

Basic rules of radiation safety

The purchase, possession, utilization, and transfer of radioactive material are subject to the regulations of the country of use. Adherence to the basic rules of radiation safety should provide adequate protection:

- No eating, drinking, smoking or application of cosmetics should be carried out in the presence of radioactive materials.
- No pipetting of radioactive solutions by mouth.
- Avoid all contact with radioactive materials by using gloves and laboratory overalls.
- All manipulation of radioactive substances should be done in an appropriate place, distant from corridors and other busy places.
- Radioactive materials should be stored in the container provided in a designated area.
- A record of receipt and storage of all radioactive products should be kept up to date.
- Laboratory equipment and glassware which are subject to contamination should be segregated to prevent cross-contamination of different radioisotopes.
- Each case of radioactive contamination or loss of radioactive material should be resolved according to established procedures.
- Radioactive waste should be handled according to the rules established in the country of use.

Sodium azide

Some reagents contain sodium azide as a preservative. Sodium azide can react with lead, copper or brass to form explosive metal azides. Sodium azide disposal must be in accordance with appropriate local regulations.

Materials of human origin

The materials of human origin, contained in this kit, were found negative for the presence of antibodies to HIV 1 and HIV 2, antibodies to HCV, as well as of Hepatitis B surface antigen (HBsAg). However, they should be handled as if capable of transmitting disease. No known test method can offer total assurance that no virus is present. Handle this kit with all necessary precautions.

All patient specimens should be handled as potentially infectious and waste should be discarded according to the country rules.

The summary of safety and performance for this in vitro diagnostic medical device is available to the public in the European database on medical device (EUDAMED) when this database is available, and the information has been uploaded by the Notified Body. The web address of the EUDAMED public web site is: https://ec.europa.eu/tools/eudamed.

To search the information about this product in EUDAMED, use BUDI-DI: 150995905IM19486P

GHS HAZARD CLASSIFICATION

Not classified as hazardous

SDS Safety Data Sheet is available at beckmancoulter.com/techdocs

SPECIMEN COLLECTION, PROCESSING, STORAGE AND DILUTION

- Serum or EDTA plasma are the recommended sample types.
- Allow serum samples to clot completely before centrifugation.
- Serum and plasma samples may be stored at 2-8°C, if the assay is to be performed within 24 hours. For longer storage keep frozen (at < -20°C, 1 year maximum), after aliquoting so as to avoid repeated freezing and thawing. Thawing of sample should be performed at room temperature.
- Dilution of samples with concentration greater than the highest calibrator is not recommended.

Serum and EDTA plasma values for 24 samples (serum values ranging from 2.67 to 12.83 U/L) were compared using the IM1948 Thymidine Kinase REA KIT. Results are as follows:

[EDTA-plasma] = 0.8055 [serum] + 0.7026

R = 0.9271

MATERIALS PROVIDED

All reagents of the kit are stable until the expiry date indicated on the kit label, if stored at 2-8°C. Expiry dates printed on vial labels apply to the long-term storage of components by the manufacturer only, prior to assembly of the kit. Do not take into account.

Once reconstituted, all the reagents should be processed. **Do not store reconstituted reagents.**

^{125}I-Tracer: one vial (lyophilized)

The vial contains 190 kBq, at the date of manufacture, of ^{125}I-labeled deoxyuridine in buffer containing bovine serum albumin and sodium azide (<0.1%).

Calibrator: one vial (lyophilized)

The calibrator vial contains Eppendorf tube with thymidine kinase with activity 80 U/L, lyophilized in buffer with bovine serum albumin and sodium azide (<0.1%). The calibrator is traceable to an internal reference standard.

Control sample: one vial (lyophilized)

The control vial contains Eppendorf tube with thymidine kinase lyophilized in human serum with sodium azide (<0.1%). The activity range is indicated on a supplement. The control sample is traceable to an internal reference standard.

Buffer: one vial (lyophilized)

The vial contains lyophilized buffer with cofactors and sodium azide (<0.1%).

BSA: one vial (lyophilized)

The vial contains lyophilized BSA with sodium azide (<0.1%).

Sorbent: one vial (powder)

Sorbent suspension in distilled water must be prepared before the assay.

MATERIALS REQUIRED, BUT NOT PROVIDED

In addition to standard laboratory equipment, the following items are required:

- Polypropylene tubes.

Use only new polypropylene tubes. Polystyrene is not suitable because high acetone concentrations lead to its structural damage and, in consequence, to discrepant results.

- Precision micropipette (25 µL).
- Semi-automatic pipette (300 µL).
- Dispensers (500 µL and 2 mL).
- Eppendorf tubes.

- Vortex type mixer.
- Aspiration system.
- Thermostat, 37°C.
- Magnetic stirrer.
- Acetone p.a., 250 mL.

Use only acetone of purity grade „puriss" or „p.a.". The use of lower grades (e.g. purum) may lead to improper sorbent decantation so that the background radioactivity reaches up to several thousands cpm and the standard curve cannot be established.

- Gamma counter set for ^{125}I.

PROCEDURE

Preparation of reagents

Let all the reagents come to room temperature.

Reconstitution of BSA

The content of the vial with lyophilized BSA is reconstituted with 2 mL of distilled water. Wait for 10 min following reconstitution and mix gently to avoid foaming before dispensing.

Preparation of working buffer solution

The content of the vial with lyophilized buffer is reconstituted with 5 mL of distilled water. Wait for 10 minutes following reconstitution and mix gently to avoid foaming. Add to a beaker with 25 mL of distilled water and then add reconstituted BSA. The total volume of this working buffer solution is 32 mL.

Reconstitution and preparation of calibrators

The content of the vial with lyophilized calibrator is reconstituted with the volume of working buffer solution indicated on the label. Wait for 10 minutes following reconstitution and mix gently to avoid foaming (see warning below this §). The enzyme activity of obtained thymidine kinase solution is 80 U/L. The other calibrators are prepared by further dilution in Eppendorf tubes. Six following levels 80, 40, 20, 10, 5, 2.5 U/L are recommended for the construction of standard curve.

Warning: Avoid foaming of the enzyme solution. It could cause enzyme denaturation. Repeating aspiration and re-addition of enzyme solution with pipette tip is satisfactory for proper mixing. Do not vortex.

Preparation of reaction mixture

The content of the tracer vial with ^{125}I-deoxyuridine is reconstituted with 30 mL of working buffer solution. Wait for 10 min following reconstitution and mix gently to avoid foaming.

Preparation of the sorbent suspension

Add the sorbent powder in 20 mL of distilled water. Prepare sorbent suspension at least 1 hour before use.

Reconstitution of control sample

The content of the vial with lyophilized control sample is reconstituted with 100 µL of working buffer solution. Wait for 10 min following reconstitution and mix gently to avoid foaming before dispensing.

Preparation of 50% acetone

Pour 250 mL of distilled water into 250 mL of acetone and homogenize.

Assay procedure

Step 1 Additions*	Step 2 Incubation
To polypropylene tubes add successively: 25 µL of working buffer solution (for calibrator 0), calibrator, control or sample and 500 µL of reaction mixture. Vortex gently 1-2 seconds.	Incubate 3.5 hours at 37°C in thermostat.

*Add 500 µL of reaction mixture to 2 additional tubes to obtain total cpm.

Step 3 Adsorption	Step 4 Counting
Add 300 µL of the sorbent suspension to all tubes (except the 2 tubes «total cpm»). Vortex gently 1-2 seconds.** Incubate 30 minutes at 18-25°C without shaking. Aspirate the supernatant so that 2-3 mm of liquid remain above the sediment. Prevent aspiration of the sediment. The determination may be interrupted at this stage and the stoppered tubes may be stored at <-18°C until the next day.	Add 2 mL of 50% acetone to all tubes (except the 2 tubes «total cpm»). Vortex briefly. Let the sorbent sediment for 5 minutes and aspirate the supernatant. Repeat the sorbent washing by 50% acetone three more times. Count bound cpm (B) and total cpm (T) for 1 minute.

**As the sorbent settles quickly, keep it in suspension to maintain its concentration during pipettation. The use of magnetic stirrer is recommended. Add sorbent suspension into each tube separately, repeated addition with dispenser is not recommended.

RESULTS

Results are obtained from the calibrator curve by interpolation. The curve serves for the determination of analyte activity in samples measured at the same time as the calibrators.

Standard curve

The results in the quality control department were calculated using *spline* curve fit with determined radioactivity *(cpm_{cal}-cpm_{cal0})* on the log vertical axis and analyte activity of the calibrators on the log horizontal axis.

Other calculation methods may give slightly different results.

Total activity: 118,187 cpm				
Calibrators	**TK (U/L)**	**cpm (n=3)**	**B/T (%)**	**cpm_{cal} – cpm_{cal0}**
0	0	220	0.19	-
1	2.5	1,501	1.27	1,281
2	5	2,426	2.05	2,206
3	10	4,081	3.45	3,861
4	20	7,399	6.26	7,179
5	40	13,840	11.71	13,620
6	80	25,105	21.24	24,885

(Example of standard curve, do not use for calculation)

Samples

For each sample, locate the cpm (cpm_{sample} - cpm_{cal0}) or B/T value on the vertical axis and read off the corresponding analyte activity on the horizontal axis.

The enzyme activity is given in units [U] per 1 litre of sample, where the unit is defined:

1 U = 1.2 x 10^{-12} mol/s

EXPECTED VALUES

It is suggested that each laboratory establishes its own normal values. The following serum values are indicative only.

Number of subjects	Average	95th percentile	99th percentile
	(U/L)		
45	3.26	6.29	10.13

QUALITY CONTROL

Good laboratory practices imply that control samples be used regularly to ensure the quality of the results obtained. These samples must be processed exactly in the same way as the assay samples, and it is recommended that their results be analyzed using appropriate statistical methods.

Failure to obtain the appropriate values for controls may indicate imprecise manipulations, improper sample handling or deterioration of reagents.

In case of packaging deterioration or if data obtained show some performance alteration, please contact your local distributor or use the following e-mail address: imunochem@beckman.com

According to EU regulation 2017/746, any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of EU Member State in which the user and/or patient is located.

PERFORMANCE CHARACTERISTICS

(For more details, see the data sheet "APPENDIX")

Representative data are provided for illustration only. Performance obtained in individual laboratories may vary.

Sensitivity

Analytical sensitivity: 0.74 U/L

Functional sensitivity: 1.87 U/L

Precision

Intra-assay

Serum samples were assayed 25 times in the same series. The coefficients of variation were found below or equal to 4.23%.

Inter-assay

Serum samples were assayed in duplicate in 10 different series. The coefficients of variation were found below or equal to 6.49%.

Accuracy

Dilution test

The dilution test is not applicable because no dilution matrix is recommended.

Measurement range (from analytical sensitivity to the highest calibrator): 0.74 to 80 U/L.

LIMITATIONS

Failure to follow these instructions for use (IFU) may significantly affect results.

Results should be interpreted in the light of the total clinical presentation of the patient, including clinical history, data from additional tests and other appropriate information.

Do not use hemolyzed, lipemic or icteric samples. For more details, see Appendix, § Interference.

Increased values can also be found e.g. in infections or inflammatory diseases. If a temporary infection is suspected, it may be necessary to repeat the test at a later occasion.

APPENDIX

PERFORMANCE CHARACTERISTICS

Representative data are provided for illustration only. Performance obtained in individual laboratories may vary.

Interference

Serum samples containing thymidine kinase enzymatic activities (low and high) were spiked with multiple concentrations of the substances listed below and assayed using Thymidine Kinase REA KIT. Values were calculated as described in CLSI EP07, 3rd ed. [4]. Interference was determined by testing controls (no interfering substance added) and matched test samples (with interfering substance added). No interference (defined as a shift in dose > 15 %) was found for addition of interferent up to concentration stated in the table below.

Interferent	Test concentration
Conjugated bilirubin	412.0 ug/mL
Hemoglobin	7,403 µg/mL
Triglycerides	22.70 mg/mL
Unconjugated bilirubin	351.3 µg/mL

In spite of hemoglobin, bilirubin (conjugated, unconjugated) and triglyceride interference data in the table, we advise to avoid using hemolyzed, lipemic or icteric samples.

Precision

Intra-assay

Serum	S1	S2	S3
Number of determinations	25	25	25
Mean value, (U/L)	6.88	18.96	53.78
C.V. (%)	4.23	4.04	3.68

EDTA plasma	P1	P2	P3
Number of determinations	25	25	25
Mean value, (U/L)	68.75	2.18	38.07
C.V. (%)	2.69	6.38	2.72

Inter-assay

Serum	S1	S2	S3
Number of determinations	10	10	10
Mean value, (U/L)	17.21	52.05	8.89
C.V. (%)	3.39	4.39	6.49

EDTA plasma	P1	P2	P3
Number of determinations	10	10	10
Mean value, (U/L)	11.28	54.16	6.55
C.V. (%)	9.01	5.76	6.49

^{125}I Characteristics

$T_{1/2}$ (^{125}I) = 1443 h = 60.14 d

^{125}I	E (MeV)	%
γ	0.035	
X	0.027	114
	0.032	25

Symbols Key

REF	Product Reference / Référence du produit / Produktreferenz / Riferimento prodotto / Número de referencia del producto / Referência do produto / Produktreferens / Κωδικός αναφοράς προϊόντος / 产品参考 / Gaminio nuoroda / Termékszám / Dane referencyjne produktu / Reference k produktu / Referenčné označenie výrobku / 제품 참조 자료 / Ürün Referansı / Ссылка на продукт / Референца за производ / 產品參考
IVD	In Vitro Diagnostic / Diagnostic in vitro / In-vitro-Diagnostikum / Diagnostica in vitro / Para diagnóstico in vitro / Diagnóstico in vitro / InVitro-diagnostik / Για διάγνωση in vitro / 体外诊断 / In vitro diagnostika / In vitro diagnosztikai felhasználásra / Diagnostyka in vitro / Diagnostika in vitro / 체외 진단 / İn Vitro Diagnostik / Диагностика in vitro / За ин витро диагностика / 體外診斷
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