MININEPH™ HUMAN β_2 MICROGLOBULIN (β_2M) KIT

For *in vitro* diagnostic use

Product Code: ZK043.L.R

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CE

1 INTENDED USE

This kit is designed for the *in vitro* measurement of human β_2Microglobulin (β_2M) in serum and urine using the MININEPH or the MININEPHPLUS*, to aid in the diagnosis of active rheumatoid arthritis and kidney disease.

**The MININEPHPLUS analyser is not available in the USA.*

2 SUMMARY AND EXPLANATION

β_2M is a low molecular weight protein (11.8kD) found on the surface of most nucleated cells. It forms the light chain component of the histocompatibility antigen. β_2M is eliminated via the kidneys, following filtration through the glomeruli it is readsorbed and catabolised by the proximal tubular cells. Normally only trace amounts are excreted in the urine - this is markedly increased in tubulo-interstitial disorders. Raised serum levels of β_2M are associated with renal disease and rheumatoid arthritis. Elevated serum levels also occur with multiple myeloma and are often raised in other types of cancer (refs 1-4).

3 PRINCIPLE OF THE ASSAY

The determination of soluble antigen concentration by nephelometric methods involves a reaction with the antibody bound to a latex particle to form insoluble complexes. When light is passed through the suspension formed, a portion of the light is scattered and detected by a photodiode. The amount of light scattered is directly proportional to the specific protein concentration in the test sample. Concentrations are automatically calculated by reference to a calibration curve stored within the instrument.

4 REAGENTS

4.1 MININEPH HUMAN β_2M REAGENT
Consisting of monospecific antiserum coated onto polystyrene microparticles. Supplied in lyophilised form. It contains 0.099% sodium azide as a preservative. Each vial should be reconstituted with 0.45mL of distilled water and allowed to stand for 30 minutes prior to use.

4.2 MININEPH β_2M SWIPE CARD
This is encoded with details of the reaction curve specific to the respective lot of reagent. This card is reagent lot specific and must be used only with this lot of reagent. The curve on this card has been prepared using secondary calibration materials that have been calibrated against 1st International Standard for beta-2-microglobin (Code: B2M), supplied by the National Institute for Biological Standards and Control (NIBSC), UK.

4.3 MININEPH β_2M BUFFER
For use with this lot of β_2M reagent only. Contains 0.099% sodium azide as a preservative.

4.4 MININEPH HUMAN β_2M HIGH AND LOW CONTROLS
These consist of pooled human serum and are supplied in stabilised liquid form. They contain 0.099% sodium azide, 0.1% EACA and 0.01% benzamidine as preservatives. The acceptable ranges of β_2M concentrations are stated on the Quality Control Certificate included in the kit. The lot number quoted on the Quality Control Certificate should be identical to the kit lot number.

5 CAUTION

All donors of human serum supplied in this kit have been serum tested and found negative for hepatitis B surface antigen (HBsAg) and antibodies to human immunodeficiency virus (HIV1 and HIV2) and hepatitis C virus. The assays used were either approved by the FDA (USA) or cleared for *in vitro* diagnostic use in the EU (Directive 98/79/EC, Annex II); however, these tests cannot guarantee the absence of infective agents. Proper handling and disposal methods should be established as for all potentially infective material, including (but not limited to) users wearing suitable protective equipment and clothing at all times. Only personnel fully trained in such methods should be permitted to perform these procedures.

WARNING: This product contains sodium azide and must be handled with caution; suitable gloves and other protective clothing should be worn at all times when handling this product. Do not ingest or allow contact with the skin (particularly broken skin or open wounds) or mucous membranes. If contact does occur wash with a large volume of water and seek urgent medical advice. Explosive metal azides may be formed on prolonged contact of sodium azide with lead and copper plumbing; on disposal of reagent, flush with a large volume of water to prevent azide build up.

This product should only be used by suitably trained personnel for the purposes stated in the Intended Use. Strict adherence to these instructions is essential at all times. Results are likely to be invalid if parameters other than those stated in these instructions are used.

Reagents from different batch numbers of kits are **NOT** interchangeable. If large numbers of tests are performed care should be taken to ensure that all reagents are from the same batch.

6 STORAGE AND STABILITY

The unopened kits should be stored at 2-8°C and can be used until the expiry date given on the kit box label. DO NOT FREEZE. The buffer should be allowed to equilibrate to room temperature prior to use. Once reconstituted the reagent is stable for 1 week. Once opened the controls should be stored at 2-8°C and are stable for 12 weeks. Once opened, the buffer should be stored at room temperature and is stable for 12 weeks. The On-Board Buffer 1 should be stored at room temperature. Opened On-Board Buffer 1 is stable for 4 weeks when stored as recommended.

7 SPECIMEN COLLECTION AND PREPARATION

7.1 SERUM SAMPLES
Blood samples should be collected by venepuncture, allowed to clot naturally and the serum separated as soon as possible to prevent haemolysis. Sera may be stored at 2-8°C for up to three days, otherwise aliquot and freeze at -20°C or below; do not freeze and thaw sera more than once. Sample dilutions should be freshly prepared on the day of assay. Some types of sera are not suitable for MININEPH assay - see section 10.1.

7.2 URINE SAMPLES
β_2M is unstable in acid urine, so the following collection procedure is recommended. The donor should void the bladder and then drink at least 0.5L of water. A urine sample should be collected within one hour, and the pH adjusted (with 1M NaOH) to pH6 - 8. Such samples can be stored for up to two days at 2-8°C, and for at least two months at -20°C or below. Repeat freezing and thawing of samples may result in deterioration and should be avoided by aliquoting prior to freezing.

8 METHODOLOGY

8.1 MATERIALS PROVIDED

8.1.1 2 x 0.45mL *MININEPH Human β_2M Reagent*
8.1.2 1 x 10mL *MININEPH β_2M Buffer*
8.1.3 1 x 0.5mL *MININEPH Human β_2M High Control*
8.1.4 1 x 0.5mL *MININEPH Human β_2M Low Control*
8.1.5 Magnetic swipe card containing lot specific calibration information
8.1.6 Quality Control Certificate
8.1.7 Instruction leaflet

8.2 MATERIALS REQUIRED BUT NOT PROVIDED (MININEPH)

8.2.1 MININEPH instrument (AD200)
8.2.2 MININEPH printer (AD210) (optional)
8.2.3 MININEPH reagent accessory pack (ZK500.R)
8.2.4 Electronic pipette (e.g. AD205)
8.2.5 A range of pipettes capable of dispensing 5 - 1000µL
8.2.6 Equipment for the collection and preparation of test samples
8.2.7 Distilled water

8.3 MATERIALS REQUIRED BUT NOT PROVIDED (MININEPHPLUS)

8.3.1 MININEPHPLUS instrument (AD500.C/D/E)
8.3.2 MININEPHPLUS PRINTER (AP1310DPK1T63) (optional)
8.3.3 Bar Code Reader (optional)
8.3.4 MININEPH reagent accessory pack (ZK500.R)
8.3.5 Pipette (5-1000µL)
8.3.6 Equipment for the collection and preparation of test samples
8.3.7 MININEPHPLUS On-Board Buffer 1 (SN107)
8.3.8 Pipette tips for use with the MININEPHPLUS - refer to MININEPHPLUS User Guide.

8.4 TEST PROCEDURE FOR MININEPH ANALYSER

8.4.1 Summary of reagent volumes added to the cuvette:

Reagent	Volume added
Sample (1/40 dilution)	30µL
MININEPH β_2M Buffer	400µL
MININEPH Hu β_2M Reagent	40µL

8.4.2 Switch the analyser and printer (if attached) on.
8.4.3 *Enter chemistry number*. Enter the chemistry number (β_2M = 43) and press **enter**.
8.4.4 *Swipe chemistry card*. This message will only be displayed if this chemistry has never been used before or you wish to change reagent lot number. Pass the swipecard through the swipecard reader moving from the front of the instrument to the back. The magnetic strip should be at the bottom facing left.
8.4.5 *Check reagent lot number*. Press **enter.**
8.4.6 *B2M lot xxxx. OK? 1=Y 2=N*. Compare the details displayed with those on the reagent label. If the lot number displayed is identical to that printed on the reagent vial, select YES (**press 1**) and continue to step 8.4.7. If the vial lot number is different from that displayed select NO (**press 2**) and return to step 8.4.4 to allow the details of the correct batch to be entered.
8.4.7 Prepare dilutions of controls and samples using the MININEPH Sample Diluent supplied in the MININEPH Reagent Accessory Pack (ZK500.R). The recommended sample dilution for β_2M in serum and urine samples is 1/40 (e.g. pipette 20µL of sample into a sample dilution tube and add 780µL of sample diluent).
8.4.8 Prepare one MININEPH cuvette for each sample to be assayed. Using the forceps provided with the MININEPH place a stirring bar in each cuvette and then using a pipette add 30µL of diluted sample carefully to the bottom of each cuvette.
8.4.9 *Enter sample ID*. Enter an identity code (e.g. 1) for the first sample to be assayed then press **enter** to continue (refer to user manual for choice of identity codes).
8.4.10 *Sample dilution 1/40.* Accept the recommended dilution by pressing **enter,** or type in a new dilution factor if an alternative dilution is to be used.
8.4.11 *Place cuvette in chamber*. Place a cuvette containing a stirring bar and 30µL of diluted sample in the cuvette chamber. Press the cuvette down gently until it reaches the bottom of the chamber. The cuvette will be detected automatically.
8.4.12 *Add reagent.* Fill an electronic pipette with 400µL of MININEPH β_2M Buffer and 40µL of MININEPH Hu β_2M Reagent and dispense its contents into the cuvette. The MININEPH will detect the addition followed by movement of the stirring bar and the assay will begin. It is not necessary to press enter. After a 30 second blanking time the assay will take 150 seconds to complete, the result will then be displayed and printed automatically (if a printer is connected).
8.4.13 If the instrument indicates the result is higher than the intended measuring range, reassay the sample at a higher dilution of 1/440 (400µL MININEPH Sample Diluent + 40µL sample diluted 1/40). The sample dilution should be entered as 1/440 (see section 8.4.10).
8.4.14 If the instrument indicates the result is lower than the intended measuring range reassay the sample at a lower dilution. The minimum recommended dilution for serum samples is 1/11 (400µL MININEPH Sample Diluent + 40 µL sample). The sample dilution should be entered as 1/11 (see section 8.4.10). The minimum recommended dilution for urine samples is 1/4 (150µL MININEPH Sample Diluent + 50µL sample). The sample dilution should be entered as 1/4 (see section 8.4.10). Use of dilutions lower than this may cause high blanks.
8.4.15 On completion of the assay remove the cuvette and press **enter** to perform the next assay.
8.4.16 When all assays for the chosen chemistry have been completed press escape (**esc**) and select the chemistry number for the next set of assays.

8.5 TEST PROCEDURE FOR MININEPHPLUS ANALYSER

8.5.1 Summary of reagent volumes added to the cuvette:

Reagent	Volume added
Sample (1/40 dilution)	30µL
MININEPH β_2M Buffer	400µL
MININEPH Hu β_2M Reagent	40µL

8.5.2 Ensure that an empty waste pot is placed at the back of the MININEPHPLUS.
8.5.3 Attach a new pipette tip on the end of the MININEPHPLUS hand held pipette.
8.5.4 Check there is sufficient On-Board buffer 1 (SN107) in the drawer. There needs to be at least 10mL. Refer to the MININEPHPLUS User Guide for instructions on replenishing the buffer.
8.5.5 Switch on the analyser and printer (if attached).
8.5.6 *Enter chemistry number.* Enter the chemistry number (β2M = 43) and press **enter**.
8.5.7 *Swipe chemistry card.* This message will only be displayed if this chemistry has never been used before or when changing reagent lot number. Pass the swipecard through the swipecard reader in a left to right direction across the front of the MININEPHPLUS with the magnetic strip facing upwards.
8.5.8 *Check reagent lot number. Press **enter**.*
8.5.9 *B2M lot xxxx. OK? 1=Y 2=N.* Compare the details displayed with those on the reagent label. If the lot number displayed is identical to that printed on the reagent vial, select YES (**press 1**) and continue to step 8.5.12. If the vial lot number is different from that displayed select NO (**press 2**) and return to step 8.5.8 to allow the details of the correct batch to be entered.
8.5.10 *Prime? 1=Y 2=N.* Prime the analyser to expel air bubbles in the plastic tube leading from the On-board buffer bottle to the hand-held pipette. This is done by pressing button **1** when prompted. Excess On-board buffer will be expelled into the waste pot. When priming has finished press **2.** Note that a prime will always be performed when starting a T1 assay that follows a T2 assay.
8.5.11 *Pipette Y/N: Block Y/N*. There is a short period when the MININEPHPLUS stabilises its temperature.
8.5.12 Prepare dilutions of controls and samples using the MININEPH Sample Diluent supplied in the MININEPH Reagent Accessory Pack (ZK500.R). The recommended sample dilution for β_2M in serum and urine samples is 1/40 (e.g. pipette 20µL of sample into a sample dilution tube and add 780µL of sample diluent).
8.5.13 Prepare one MININEPH cuvette for each sample to be assayed. Using the forceps provided with the MININEPHPLUS place a stirring bar in each cuvette and then using a pipette add 30µL of diluted sample carefully to the bottom of each cuvette.
8.5.14 *Enter sample ID.* Enter an identity code (e.g. 1) for the first sample to be assayed then press **enter** to continue (refer to user manual for choice of identity codes).
8.5.15 *Sample dilution 1/40.* Accept the recommended dilution by pressing **enter** or type in a new dilution factor if an alternative dilution is to be used.
8.5.16 *Place cuvette in chamber.* Place a cuvette containing a stirring bar and 30µL of diluted sample in the cuvette chamber. Press the cuvette down gently until it reaches the bottom of the chamber. The cuvette will be detected automatically.
8.5.17 *Supplementary buffer.* Using the MININEPHPLUS hand-held pipette, aspirate 400µL of MININEPH β_2M buffer.
8.5.18 *Air Gap.* Using the MININEPHPLUS hand-held pipette, aspirate an air gap.
8.5.19 *Aspirate Reagent* Using the MININEPHPLUS hand-held pipette, aspirate 40µL of MININEPH Human β_2M reagent.
8.5.20 *Add Reagent.* Dispense the aspirated reagents into the cuvette. The stirring bar will rotate and the assay will begin. After a 30 second blanking time the assay will take 148 seconds to complete. The result will be displayed. Results will be automatically printed if a printer is connected.
8.5.21 If the instrument indicates the result is higher than the intended measuring range, reassay the sample at a higher dilution of 1/440 (400µL MININEPH Sample Diluent + 40µL sample diluted 1/40). The sample dilution should be entered as 1/440 (see section 8.5.15).
8.5.22 If the instrument indicates the result is lower than the intended measuring range reassay the sample at a lower dilution. The minimum recommended dilution for serum samples is 1/11 (400µL MININEPH Sample Diluent + 40µL sample). The minimum recommended dilution for urine samples is 1/4 (150µL MININEPH Sample Diluent + 50µL sample). The sample dilution should be entered as 1/4 (see section 8.4.10). Use of dilutions lower than this may cause high blanks. The sample dilution should be entered as 1/11 (see section 8.5.15).
8.5.23 On completion of the assay remove the cuvette and press **enter** to perform the next assay.
8.5.24 When all assays for the chosen chemistry number have been completed press **esc** and select the chemistry number for the next set of assays.
8.5.25 Empty waste pot and discard the pipette tip from the hand held pipette.

8.6 QUALITY CONTROL

As with all good laboratory practice, users should run controls with every batch of samples. If controls fall outside of the range quoted on the Quality Control Certificate see Section 10.2 below. If problems persist please refer to supplier.

9 INTERPRETATION OF RESULTS

9.1 Results are calculated by the instrument and displayed in mg/L. If a printer is attached the result is automatically printed out together with the patient identification code and the sample dilution. Further calculations are not necessary.
9.2 The approximate measuring range is 0.75 - 12.0mg/L at the recommended sample dilution of 1/40. The sensitivity limit for serum samples is 0.2mg/L using a 1/11 sample dilution, and 0.075mg/L for urine samples using a 1/4 sample dilution. Sample concentrations up to at least 80mg/L will not result in antigen excess. Higher concentrations may give misleading results, if this is suspected, samples should be assayed at a 1/440 dilution (400µL MININEPH Sample Diluent + 40µL sample diluted 1/40).

10 LIMITATIONS OF PROCEDURE

10.1 SPECIFIC TEST LIMITATIONS

10.1.1 Nephelometric assays are not suitable for measurement of highly lipaemic or haemolysed samples or samples containing high levels of circulating immune complexes (CICs) due to the unpredictable degree of non-specific scatter these sample types may generate. Unexpected results should be confirmed using an alternative assay method.
10.1.2 Diagnosis cannot be made and treatment must not be initiated on the basis of β_2M measurements alone. Clinical history and other laboratory findings must also be taken into account.

10.2 TROUBLE SHOOTING

Problem	Possible Cause(s)	Suggested Action(s)
Error message "Blank too high - reassay" displayed.	Very high analyte concentration.	Reassay sample at a higher dilution.
	Turbid samples.	Try alternative assay method.
Controls out of range.	Product deterioration.	Check expiry date.
	Operator error.	Repeat assay with the correct sample dilution.
Test sample giving unexpectedly low result.	Antigen excess.	Repeat assay at higher dilution. Check if the two results agree.

11 EXPECTED RESULTS

The following β_2M results were obtained with normal adult donor sera and urine samples on the BNII analyser. Concentrations are in mg/L. We recommend local reference ranges are generated.

Sample Type	Number	Mean	95% Range
Serum	150	1.65	1.22 -2.46
Urine	55	-	<0.03-0.23

12 PERFORMANCE CHARACTERISTICS

12.1 PRECISION

12.1.1 Precision - MININEPH

β_2M Precision Summary				
	Mean mg/L	Intra batch CV% (n=30*)	Day to day CV% (n=30**)	Inter instrument CV% (n=15***)
Serum 1	4.6	2.3	6.6	4.0
Serum 2	2.1	4.4	5.6	5.5

12.1.2 Precision - MININEPHPLUS

β_2M Precision Summary				
	Mean mg/L	Intra batch CV% (n=30*)	Day to day CV% (n=30**)	Inter instrument CV% (n=15***)
Serum 1	4.3	3.6	5.7	6.6
Serum 2	1.9	3.8	5.2	4.9

**These data represent the average coefficient of variation (CV) of three within-batch measurements repeated ten times at each concentration.*
***Ten within-batch measurements were performed on three separate occasions and the overall CV for the thirty results at each concentration calculated.*
****Assays were performed three times at each concentration on five instruments. The overall CV of the fifteen results at each concentration was calculated.*

12.2 COMPARISON STUDIES

12.2.1 MININEPH

12.2.1.1 SERUM SAMPLES: A correlation study was performed on 49 normal and 50 clinical serum samples using this kit on the MININEPH and a Binding Site β_2M kit on the BN™A analyser. The study demonstrated a good agreement yielding the following linear regression equation and correlation coefficient.

y = 0.960x + 0.02 mg/L (y = MININEPH β_2M)
(x = BNA β_2M)

correlation coefficient r = 0.991

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A correlation study was performed on 18 serum samples using this kit on the MININEPH and a Binding Site β_2M RID kit. The study demonstrated a good agreement yielding the following linear regression equation and correlation coefficient.

y = 0.925x + 0.32 mg/L (y = MININEPH β_2M)
(x = RID β_2M)

correlation coefficient r = 0.993

12.2.1.2 URINE SAMPLES: A correlation study was performed on 22 normal and 29 clinical urine samples using this kit on a MININEPH and a Binding Site β_2M kit on the BNA. The study demonstrated good agreement yielding the following linear regression equation and correlation coefficient.

y = 1.021x - 0.001 mg/L (y = MININEPH β_2M)
(x = BNA β_2M)

correlation coefficient r = 0.987

12.2.2 MININEPHPLUS

21 adult sera were tested on the MININEPH and MININEPHPLUS. The study demonstrated a good agreement yielding the following Passing & Bablok equation and linear regression correlation coeffiecient:

y = 1.09x - 0.01mg/L (y = MININEPHPLUS β_2M)
(x = MININEPH β_2M)

correlation coefficient r = 0.996

21 clinical urine samples were tested on the MININEPH and MININEPHPLUS. The study demonstrated a good agreement yielding the following Passing & Bablok equation and linear regression correlation coeffiecient:

y = 0.94x + 0.10mg/L (y = MININEPHPLUS β_2M)
(x = MININEPH β_2M)

correlation coefficient r = 0.996

13 REFERENCES

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3. Crisp, AJ *et al.* (1983). Beta-2 microglobulin plasma levels reflect activity in rheumatoid arthritis. J. Rheumatol. 10, 954-956.
4. Protein Reference Unit Handbook of clinical Immuno Chemistry (1999) Ed. A Milford Ward et al, Publ. PRU Publications, Sheffield, 53-56.