



ALS RIA KIT

REF	10 ALS50	REF	10 ALS100
Σ	50	Σ	100



WARRANTY

The manufacturer makes no express warranty other than the diagnostic kit will measure the designated analyte when used in accordance with the manufacturer's printed instructions. The use of the diagnostic kit for any other purpose is outside the intended use of this product and is done at the user's own risk.

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INTENDED USE

The ALS RIA has been designed for the quantitative *in vitro* diagnostic measurement of ALS (acid-labile subunit) in serum or plasma.

PRINCIPLES OF THE RIA

The RIA is a double antibody radioimmunoassay system. The analyte in diluted samples and calibrators competes with ¹²⁵I labelled tracer antibody for binding to a constant amount of antibody.

A second antibody coupled to magnetisable polystyrene particles (Separation Reagent) is used to separate antibody-bound from free ¹²⁵I labelled tracer antibody. Following sedimentation, the supernatant is discarded and the pellet containing the bound radioactivity is counted using a gamma counter. The concentration of the analyte is inversely proportional to the bound radioactivity in the pellet. Counts from the calibrators are plotted and samples are read from the constructed calibrator curve.

REAGENTS PROVIDED, STABILITY AND STORAGE

Kit size - 50 tests and 100 tests (in parentheses). The kit and all its components should be stored at 2-8°C until the listed expiry dates.

ALS: Tracer

1 vial REF # ALI1
(1 vial REF # ALI2)
 5.5 (10.5) mL ¹²⁵I des labelled ALS ($\leq 40\text{kBq}$) in BSA PBS buffer containing a red dye. Contains sodium azide, (NaN₃), 0.1% w/v. Ready to use.

ALS: Antiserum

1 vial REF # ALA1
(1 vial REF # ALA2)
 5.5 (10.5) mL containing rabbit ALS antiserum diluted in BSA PBS buffer and a blue dye. Contains NaN₃, 0.1% w/v. Ready to use.

Separation Reagent

1 vial REF # SEPI
(1 vial REF # SEP2)
 13 (26) mL containing goat anti-rabbit antibody coupled to magnetisable polystyrene particles in BSA PBS buffer. Contains NaN₃, 0.1% w/v. Resuspend gently before use.

ALS: Calibrators

1 vial REF # ZC1
(1 vial REF # ZC2)
 25 (50) mL each of Calibrator A (0 nmol/L concentrate), containing a 4 x concentrated solution of BSA PBS buffer. Contains NaN₃, 0.4% w/v.

To be diluted before use.

5 vials REF # ALS2-6
 1.0 mL each of Calibrator B-F in BSA PBS buffer. Contains NaN₃, 0.1% w/v. Lyophilized.

ALS: Controls

2 vials REF # ALC1-2
 1.0 mL each in BSA PBS buffer. Contains NaN₃, 0.1% w/v. Lyophilized.

Do not dilute.

PRECAUTIONS AND WARNINGS TO USERS

Handling of specimens and kit components, their use, storage and disposal should be in accordance with any local or national laboratory safety procedures or regulations.

Specimens, Calibrators and Controls

The source material of the calibrators and controls has been tested by an approved accredited method for the presence of hepatitis B surface antigen, antibody to hepatitis C and antibody to HIV - 1/2 (AIDS) and has been found to be non-reactive for all. However it is recommended that all samples be handled as if capable of transmitting infectious disease.

Preservatives

The kit contains sodium azide as a preservative. As reagents contain a potentially toxic preservative, care should be taken in handling, to avoid ingestion or skin contact. Sodium azide may react with lead and copper plumbing to form potentially explosive azides.

Radioactive Material

The tracer contains radioactive material.

SPECIMEN COLLECTION AND HANDLING

No special patient preparation is required. Specimens can be either serum or plasma collected in a manner appropriate for laboratory testing. Serum is preferred, however the anticoagulants heparin or EDTA can be employed without sacrificing accuracy.

Avoid grossly haemolytic, lipaemic and turbid specimens. Specimens can be stored at 2-8°C for up to 48 hours. Specimens held for longer should be stored at or below -20°C. Specimens should not be frozen and thawed repeatedly.

Thawed specimens should be checked for flocculent matter and mixed by inversion just prior to testing. Turbid specimens or specimens containing particulate matter should be centrifuged prior to use.

MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- * Distilled and deionised water
- * Disposable plastic test tubes with caps 12 x 75 mm
- * Precision pipettes
- * Repeating pipettes
- * Vortex mixer
- * Roller Bench
- * Timer
- * Magnetic Rack
- * Absorbent paper
- * Gamma counter

PROCEDURAL NOTES

Bring all reagents and specimens to room temperature (20-25°C) and mix by gentle inversion prior to use. duplicates are recommended. Contamination of reagents will lead to poor performance. A calibrator curve should be run with each assay. [$>$ Top calibrator, to be diluted in zero]. All assay steps should be performed without interruption.

Reagents are matched in each kit and therefore reagents from different lot numbers should not be mixed.

The gamma counter and all pipettes used should be calibrated appropriately before use.

Quality Control

Control specimens should be run in every assay to ensure correct procedure. Control values should lie within laboratory ranges before assay is approved.

ASSAY PROCEDURE

Preparation of Reagents Calibrators and Controls

To reconstitute the lyophilized calibrators and controls, add the volume of deionized water indicated on each vial label. Allow the vials to sit undisturbed until completely dissolved (at least 30 minutes) and then mix by gentle inversion. Exact concentrations determined lot-to-lot are stated on a separate label inside the kit.

After reconstitution the calibrators and controls can be stored at -20°C.

Dilution Procedure

Calibrator A

0 nmol/L Concentrate

Dilute Calibrator A, 1 in 4 with deionised water. If the Calibrator A has crystallised, warm to 37°C. The Calibrator A solution is then also used as the sample diluent and can be stored at 2-8°C until the listed expiry date.

Sample Preparation

Samples (not calibrators/controls) should be diluted 1 in 200.

1. Label dilution tubes (1 per sample)

- Pipette 10µL sample, add 1.99 mL of Calibrator A diluent. Vortex.

Separation Reagent

Mix well on a roller bench before use.

Protocol

- Assemble and label test tubes in duplicate according to the number of tests required. Include Total Counts (TC), Non-Specific Binding (NSB), calibrators, extracted controls/ specimens.
- Pipette 200 µL of Calibrator A in duplicate into the NSB tubes.
- Pipette 100 µL of sample (calibrator, control, specimen) in duplicate into the appropriate tubes.
- Pipette 100 µL of ALS Antiserum (blue) to all tubes except NSB and TC.
- Pipette 100 µL of ALS Tracer (red) into all tubes.
- Vortex tubes gently and incubate overnight (16-24 hours) stationary at room temperature (20-25°C). All tubes should be purple except NSB and TC tubes.

CALCULATION OF RESULTS

Calculation of results can be carried out manually if there is no automatic data reduction. Calibrators are established to allow for a sample dilution of 1 in 200.

- Determine the average cpm for duplicate tubes.
- Plot the calibrator curve on a semi-log or log-linear graph paper using the method below:

Use the following formula to calculate %B/T:

$$\%B/Bo = \frac{\text{cpm (Sample)} - \text{cpm (NSB)}}{\text{cpm (Calibrator A)} - \text{cpm (NSB)}} \times 100$$

- Plot %B/Bo on the y axis versus the stated concentrations of the calibrators.
- Read samples directly off the calibrator curve as nmol/L.

MODEL CALCULATIONS

ID	Ave cpm	%B/Bo	ALS (nmol/L)
TC	24059		
NSB	373		
0	11622	100.0	
10	10010	85.7	
30	8349	70.9	
100	5107	42.1	
300	2665	20.4	
1000	1276	8.0	
Control 1	7130	63.4	49.0
Control 2	3516	27.9	196.0
Sample 1	5122	42.2	102.0

CALIBRATION

The calibrators supplied in this kit are calibrated to highly purified ALS (M.W.:63,300; referenced by amino acid analysis). They are labelled in nmol/L.

Conversion of calibrator units may be made using the following relationship:

$$1.0 \mu\text{g/mL ALS} = 15.8 \text{ nmol/L ALS}$$

LIMITATIONS

Serum samples showing gross haemolysis, gross lipaemia, or turbidity may give false results.

Samples that contain appreciable background radioactivity should not be used. Any suspect samples should be screened for radioactivity before performing the assay and should be held until the radioactivity has decayed, or a new sample requested.

- At the end of the incubation period, pipette 250 µL of the thoroughly mixed Separation Reagent into all tubes except TC and vortex. Set TC tubes aside, and incubate for 15 minutes stationary at room temperature (20-25°C).

8a. To Separate antibody from unbound label, place test tubes into magnetic separation rack and ensure all test tubes are in contact with magnetic baseplate. Leave for 2 minutes.

8b. Do not remove rack from magnetic baseplate. Decant the supernatant and keep magnetic baseplate inverted. Tap the tubes firmly onto absorbent paper and blot the rims to remove all residual supernatant.

- Count the tubes for one minute using a gamma counter. Counting longer will reduce statistical counting error.

Record the cpm of each tube.

- Calculate results.

EXPECTED VALUES

It is recommended that each laboratory establish its own reference range based on a representative sample population. The following reference range was obtained by assaying serum samples from healthy individuals and is given as a guide only:

Age (Yrs)	n	Mean	SD	Median	5th Percentile	95th Percentile
(Male & Female)	30	80.2	62.4	72.4	17.7	179.0
1 - 3	45	103.0	58.1	103.0	33.6	171.0
4 - 6	34	123.0	67.1	133.0	6.1	240.0
7 - 9	43	173.0	107.0	166.0	77.2	297.0
10 - 12	40	222.0	150.0	241.0	117.0	309.0
13 - 15	54	261.0	140.0	267.0	132.0	350.0
16 - 18	27	291.0	109.0	296.0	200.0	369.0
19 - 30	104	223.0	96.7	226.0	130.0	301.0
31 - 40	55	211.0	75.1	208.0	114.0	296.0
41 - 50	76	203.0	65.2	208.0	109.0	279.0
51 - 60	57	182.0	58.9	178.0	88.1	259.0
61+	18	172.0	54.6	171.0	87.1	241.0

PERFORMANCE CHARACTERISTICS

Intra-assay Precision

Sample	n	Mean ± 2SD (nmol/L)	%CV
A	22	26.1 ± 1.4	5.0
B	22	48.5 ± 2.6	5.4
C	22	207.0 ± 5.8	2.8
D	22	488.0 ± 20.0	4.1

Inter-assay Precision

Sample	n *	Mean ± 2SD (nmol/L)	%CV
E	39	50.6 ± 2.1	4.2
F	39	104.0 ± 3.6	3.5
G	39	176.0 ± 6.9	3.9
H	39	208.0 ± 8.2	3.9
I	39	344.0 ± 16.7	4.9

* duplicate

Specificity

Analyte	Concentration Assayed	Apparent ALS Result (nmol/L)
IGFBP-1	1000 ng/mL	undetectable
IGFBP-2	1000 ng/mL	undetectable
IGFBP-3	100 µg/mL	undetectable
IGF-I	4000 ng/mL	undetectable
IGF-II	4000 ng/mL	undetectable

Accuracy

Recovery was calculated by assaying before and after addition of exogenous analyte.

Sample	ALS (nmol/L) Observed	ALS (nmol/L) Expected	% Recovery
1	57.6	55.4	104.0
2	75.3	73.8	102.0
3	296.0	292.0	101.3
4	580.0	568.0	102.1

Dilution

A sample was diluted in zero calibrator, assayed and recovery calculated.

Sample	ALS (nmol/L) Observed	ALS (nmol/L) Expected	% Recovery
Neat	446.0		
1/2	242.0	223.0	109.0
1/4	116.0	112.0	104.0
1/8	59.1	55.8	106.0
1/16	25.9	27.9	92.8

Sensitivity

The sensitivity is defined as the concentration of analyte corresponding to two standard deviations greater than the mean of the zero binding (measured in 9 assays) is 4 nmol/L. In terms of the actual concentration of ALS, the sensitivity is 0.02 nmol/L.

Interference

No interference with analyte recovery was observed for concentrations of haemoglobin up to 250 mg/dL, bilirubin up to 10 mg/dL and triglycerides up to 970 mg/dL.

ORDERING INFORMATION

The ALS RIA is manufactured by:

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