

DuoSet[®] IC

Human/Mouse/Rat Total GSK-3 α / β

Catalog Number DYC2157-2

For the development of sandwich ELISAs to measure GSK-3 α / β in cell lysates.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

TABLE OF CONTENTS

SECTION	PAGE
PRINCIPLE OF THE ASSAY.....	1
MATERIALS PROVIDED & STORAGE CONDITIONS	1
OTHER MATERIALS REQUIRED	2
SOLUTIONS REQUIRED.....	2
REAGENT PREPARATION.....	3
PREPARATION OF SAMPLES.....	3
PRECAUTIONS.....	4
TECHNICAL HINTS AND LIMITATIONS.....	4
GENERAL ELISA PROTOCOL	5
CALCULATION OF RESULTS	6
TYPICAL DATA.....	6
CALIBRATION	6
SPECIFICITY.....	7
QUANTIFICATION	7
PLATE LAYOUT	9

MANUFACTURED AND DISTRIBUTED BY:

USA & Canada | R&D Systems, Inc.

614 McKinley Place NE, Minneapolis, MN 55413, USA
TEL: (800) 343-7475 (612) 379-2956 FAX: (612) 656-4400
E-MAIL: info@RnDSystems.com

DISTRIBUTED BY:

UK & Europe | R&D Systems Europe, Ltd.

19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

China | R&D Systems China Co., Ltd.

24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
TEL: +86 (21) 52380373 FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

PRINCIPLE OF THE ASSAY

This DuoSet® IC ELISA contains the basic components required for the development of sandwich ELISAs to measure the amount of Glycogen Synthase Kinase-3 alpha and beta (GSK-3 α / β) in cell lysates. An immobilized capture antibody specific for GSK-3 α and GSK-3 β binds both phosphorylated and unphosphorylated protein. After washing away unbound material, a biotinylated detection antibody specific for GSK-3 α and GSK-3 β is used to detect captured protein, utilizing a standard Streptavidin-HRP format.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

DESCRIPTION	PART #	CATALOG # DYC2157-2	CATALOG # DYC2157-5	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human/Mouse/Rat GSK-3 α / β Capture Antibody	841938	1 vial	2 vials	Store for up to 1 month at 2-8 °C or aliquot and store at ≤ -20 °C in a manual defrost freezer or at ≤ -70 °C for up to 3 months.*
Human/Mouse/Rat GSK-3 α / β Detection Antibody	841939	1 vial	2 vials	
Human/Mouse/Rat GSK-3 α / β Standard	841940	3 vials	5 vials	Use within one hour of reconstitution. Use a fresh standard for each assay.
Streptavidin-HRP	890803	1 vial	1 vial	Store at 2-8 °C. DO NOT FREEZE.

* Provided this is within the expiration date of the kit.

DYC2157-2 contains sufficient materials to run ELISAs on at least two 96 well plates.[†]

DYC2157-5 contains sufficient materials to run ELISAs on at least five 96 well plates.[†]

[†] Provided the following conditions are met:

- The reagents are prepared as described in this package insert.
- The assay is run as described in the General ELISA Protocol on page 5.
- The recommended microplates, buffers, diluents, substrates, and solutions are used.

OTHER MATERIALS REQUIRED

- Aprotinin (Sigma # A6279)
- Leupeptin (Tocris # 1167)
- Pepstatin (Tocris # 1190)
- Phenylmethylsulfonylfluoride (PMSF) (Sigma # P7626)
- Sodium Azide (NaN_3) (Sigma # S2002)
- Sodium Fluoride (NaF) (Sigma # 201154)
- Sodium Orthovanadate (Na_3VO_4) (Sigma # S6508), activated
- Sodium Pyrophosphate ($\text{Na}_4\text{P}_2\text{O}_7$) (Sigma # P8010)
- Triton[®] X-100 (Sigma # T9284)
- Urea
- Pipettes and pipette tips
- Deionized or distilled water
- 96 well microplates (R&D Systems[®], Catalog # DY990)
- Plate sealers (R&D Systems[®], Catalog # DY992)
- Squirt bottle, manifold dispenser, or automated microplate washer

SOLUTIONS REQUIRED

PBS - 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4 , 1.5 mM KH_2PO_4 , pH 7.2-7.4, 0.2 μm filtered. (R&D Systems[®], Catalog # DY006).

Wash Buffer - 0.05% Tween[®] 20 in PBS, pH 7.2-7.4 (R&D Systems[®], Catalog # WA126).

Block Buffer - 1% BSA*, 0.05% NaN_3 , in PBS, pH 7.2-7.4.

IC Diluent #1 - 1% BSA* in PBS, pH 7.2-7.4, 0.2 μm filtered. (R&D Systems[®], Catalog # DY995)

IC Diluent #8** - 1 mM EDTA, 0.5% Triton X-100, 5 mM NaF in PBS, pH 7.2-7.4.

Note: IC Diluent #8 is also the base diluent for IC Diluent #3, IC Diluent #7, and Lysis Buffer #6. Approximately 50 mL of this diluent is required to run the assay on one plate.

IC Diluent #3** - 1 mM EDTA, 0.5% Triton X-100, 5 mM NaF, 1 M urea in PBS, pH 7.2-7.4.

IC Diluent #7** - 1 mM EDTA, 0.5% Triton X-100, 5 mM NaF, 6 M urea in PBS, pH 7.2-7.4.

Lysis Buffer #6** - 1 mM EDTA, 0.5% Triton X-100, 5 mM NaF, 6 M urea, 1 mM activated sodium orthovanadate, 2.5 mM sodium pyrophosphate, 10 $\mu\text{g}/\text{mL}$ Leupeptin, 10 $\mu\text{g}/\text{mL}$ Pepstatin, 100 μM PMSF, and 3 $\mu\text{g}/\text{mL}$ Aprotinin in PBS, pH 7.2-7.4.

Substrate Solution - 1:1 mixture of Color Reagent A (H_2O_2) and Color Reagent B (Tetramethylbenzidine) (R&D Systems[®], Catalog # DY999).

Stop Solution - 2 N H_2SO_4 (R&D Systems[®], Catalog # DY994).

*The use of R&D Systems[®] Reagent Diluent Concentrate 2 (Catalog # DY995) or Millipore Bovine Serum Albumin, Fraction V, Protease free (Catalog # 82-045) is recommended. All buffers containing BSA must be stored at 2-8 °C.

**Alternatively, use Sample Diluent Concentrate 1 (5X) (R&D Systems[®], Catalog # DYC001), prepared as described in the DYC001 insert.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Human/Mouse/Rat GSK-3 α / β Capture Antibody (Part 841938) - Each vial contains 360 μ g/mL of rabbit anti-human GSK-3 α / β antibody when reconstituted with 200 μ L of PBS.

Human/Mouse/Rat GSK-3 α / β Detection Antibody (Part 841939) - Each vial contains 3.6 μ g/mL of biotinylated rabbit anti-human GSK-3 α / β antibody when reconstituted with 1.0 mL of IC Diluent #1.

Human/Mouse/Rat GSK-3 α / β Standard (Part 841940) - Each vial contains 200 ng/mL of recombinant human GSK-3 α when reconstituted with 500 μ L of IC Diluent #7. Immediately before use, an initial 6-fold dilution should be made in IC Diluent #8. Additional dilutions should be made in IC Diluent #3. A seven point standard curve using 2-fold serial dilutions and a high standard of 20,000 pg/mL is recommended.

Streptavidin-HRP (Part 890803) - 1.0 mL of Streptavidin conjugated to horseradish-peroxidase. Immediately before use, dilute the Streptavidin-HRP to the working concentration specified on the vial label using IC Diluent #1.

PREPARATION OF SAMPLES

Cell Lysates - Rinse cells two times with PBS, making sure to remove any remaining PBS after the second rinse. Solubilize cells at 1×10^7 cells/mL in Lysis Buffer #6 and allow samples to sit on ice for 15 minutes. Assay immediately or store at ≤ -70 °C. Before use, centrifuge samples at 2000 x g for 5 minutes and transfer the supernate to a clean test tube. Sample protein concentration may be quantified using a total protein assay. For assaying, dilute lysates 6-fold with IC Diluent #8 and make further serial dilutions in IC Diluent #3.

Note: *The final concentration of urea in all samples and standards should be 1 M prior to addition to the plate.*

PRECAUTIONS

The Stop Solution recommended for use with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B recommended for use with this kit may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the MSDS on our website prior to use.

TECHNICAL HINTS AND LIMITATIONS

- This DuoSet® IC ELISA should not be used beyond the expiration date on the kit label.
- Individual results may vary due to differences in technique, plasticware, and water sources.
- It is important that the diluents selected for reconstitution and for dilution of the standard reflect the environment of the samples being measured. The diluent suggested in this protocol should be suitable for most cell lysates.
- The type of enzyme and substrate and the concentrations of capture/detection antibodies used can be varied to create an immunoassay with a different sensitivity and dynamic range. A basic understanding of immunoassay development is required for the successful use of these reagents in immunoassays.
- A thorough and consistent wash technique is essential for proper assay performance. Wash Buffer should be dispensed forcefully and removed completely from the wells by aspiration or decanting. Remove any remaining Wash Buffer by inverting the plate and blotting it against clean paper towels.
- Use a fresh reagent reservoir and pipette tips for each step.
- It is recommended that all standards and samples be assayed in duplicate.
- Avoid microbial contamination of reagents and buffers. This may interfere with the sensitivity of the assay. Buffers containing protein should be made under aseptic conditions and stored at 2-8 °C or be prepared fresh daily.

GENERAL ELISA PROTOCOL

A plate layout is provided to record standards and samples assayed.

Plate Preparation

1. Dilute the capture antibody to a working concentration of 2.0 µg/mL in PBS, without carrier protein. Immediately coat a 96 well microplate with 100 µL per well of the diluted capture antibody. Seal the plate and incubate overnight at room temperature.
2. Aspirate each well and wash with Wash Buffer, repeating the process two times for a total of 3 washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.
3. Block plates by adding 300 µL of Block Buffer to each well. Incubate at room temperature for 1-2 hours.
4. Repeat the aspiration/wash as in step 2. The plates are now ready for sample addition.

Assay Procedure

1. Add 100 µL of sample or standards in IC Diluent #3 per well. Use IC Diluent #3 as the zero standard. Cover with a plate sealer and incubate 2 hours at room temperature.
Note: *A seven point standard curve using 2-fold serial dilutions and a high standard of 20,000 ng/mL is recommended.*
2. Repeat the aspiration/wash as in step 2 of Plate Preparation.
3. Dilute the detection antibody to a working concentration of 100 ng/mL in IC Diluent #1 immediately before use. Add 100 µL of the diluted detection antibody to each well. Cover with a new plate sealer and incubate 2 hours at room temperature.
4. Repeat the aspiration/wash as in step 2 of Plate Preparation.
5. Add 100 µL of the diluted Streptavidin-HRP to each well. Incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
6. Repeat the aspiration/wash as in step 2 of the Plate Preparation.
7. Add 100 µL of Substrate Solution to each well. Incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
8. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well immediately, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

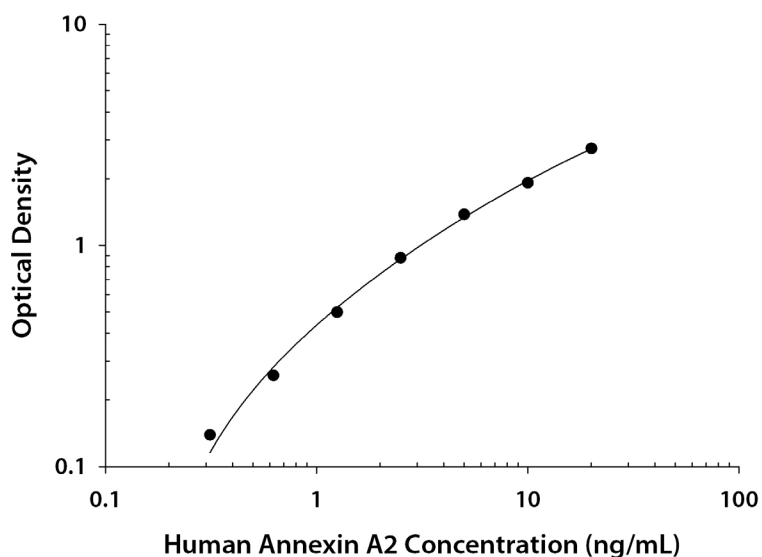
CALCULATION OF RESULTS

Average the duplicate readings for each standard and sample, then subtract the average zero standard optical density (O.D.). Results may be normalized to total protein or cell number.

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the GSK-3 α / β concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

TYPICAL DATA

A standard curve should be generated for each set of samples assayed. The graph below represents typical data generated when using this Human Total GSK-3 α / β DuoSet[®] IC ELISA. The standard curve was calculated using a computer generated 4-PL curve-fit. This standard curve is for demonstration purposes only.



CALIBRATION

The Human/Mouse/Rat Total GSK-3 α / β DuoSet[®] IC ELISA is calibrated against a highly purified *E. coli*-expressed recombinant human GSK-3 α produced at R&D Systems[®]. Samples containing natural GSK-3 α / β showed linear dilution parallel to the standard curve obtained using the Total GSK-3 α / β Standard. These results indicate that O.D. values from this DuoSet[®] IC ELISA can be used to determine the concentrations of GSK-3 α / β in natural samples.

SPECIFICITY

To determine specificity, recombinant human (rh) ERK1 and rhp38 α , were assayed at 200 ng/mL and read as 2.2 ng/mL (7.4% cross-reactivity) and 1.7 ng/mL (4.5% cross-reactivity), respectively. Recombinant human Akt1 and rhAkt2 were assayed at 200 ng/mL and did not cross-react or interfere in the assay.

QUANTIFICATION

Amounts of human GSK-3 α and β , as quantified by the Total GSK-3 α/β DuoSet[®] IC ELISA, are consistent with the relative amounts of GSK-3 isoforms determined by qualitative Western Blot analysis.

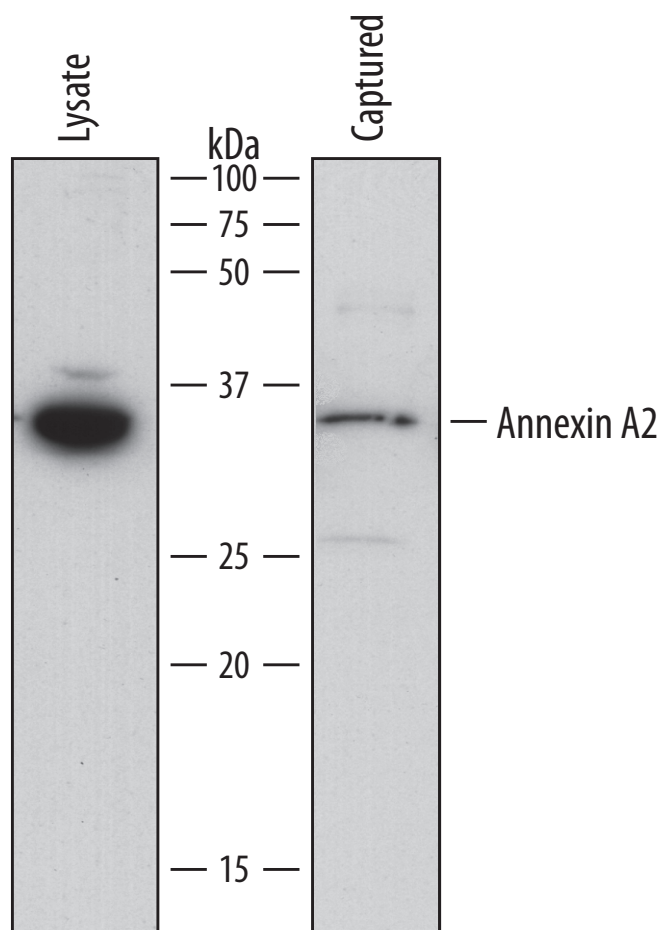


Figure 1: Lysates prepared from MCF-7 human breast cancer cells, KG-1 human acute myelogenous leukemia cells, HeLa human cervical epithelial carcinoma cells, THP-1 human acute monocytic leukemia cells, HepG2 human hepatocellular carcinoma cells, and HT-29 human colon adenocarcinoma cells were quantified with this DuoSet[®] IC ELISA. The same lysates were also immunoblotted (inset) with anti-GSK-3 α/β polyclonal antibody (R&D Systems[®], Catalog # AF2157). The DuoSet[®] IC ELISA results correlate well with the relative amounts of GSK-3 α (upper band) and GSK-3 β (lower band) detected by Western Blot.

The Total GSK-3a/b DuoSet IC ELISA also quantifies GSK-3 isoform levels in mouse and rat cell lysates.

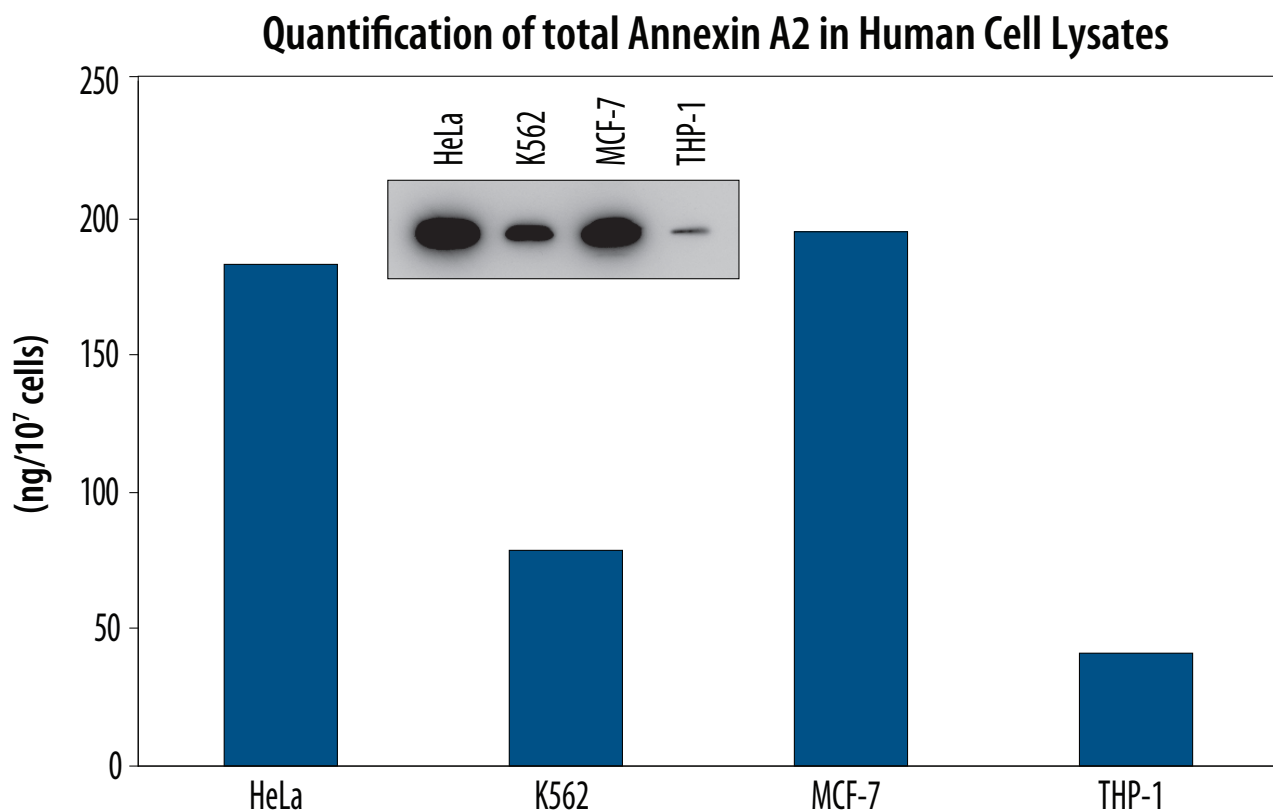


Figure 2: Lysates prepared from NIH-3T3 mouse embryonic fibroblast cells, CTLL-2 mouse cytotoxic T cells, L-929 mouse fibroblast cells, DA3 mouse myeloma cells, C2C12 mouse myoblast cells, and PC-12 rat adrenal pheochromocytoma cells were quantified with this DuoSet IC ELISA. The same lysates were also immunoblotted (inset) with anti-GSK-3a/b polyclonal antibody. The DuoSet IC ELISA results correlate well with the relative amounts of GSK-3a and b detected by Western blot.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.

12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1	A	B	C	D	E	F	G	H

NOTES

All trademarks and registered trademarks are the property of their respective owners.

©2017 R&D Systems®, Inc.

10.05

751544.3

1/17

10

For research use only. Not for use in diagnostic procedures.