Multi-Kinase ELISA Array

Human/Mouse/Rat

GSK3α (pS21) & GSK3β (pS9) ELISA

Instruction Manual

For the qualitative detection of phosphorylated GSK3α & GSK3β in tissue lysates, cell lysates and recombinant protein preparations.

MKA 003

Version 5

For Research Use Only
The kit should be stored at 4°C upon receipt.

www.symansis.com

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Introduction

Glycogen synthase kinase-3 (GSK-3) is a ubiquitously expressed serine/threonine protein kinase that phosphorylates and inactivates glycogen synthase. GSK-3 is a critical downstream element of the PI3 kinase/Akt signal transduction pathway, and its activity can be inhibited by Akt-mediated phosphorylation at Ser21 of GSK-3α and Ser9 of GSK-3β GSK-3 is also a key component of the Wnt signaling pathway. Symansis has formulated a **Denaturing Cell Lysis buffer (cat # CLB001)** containing 6M Urea to maximize assay performance. It is strongly recommended that this buffer be used; assay performance using lysis buffers without urea will be less effective.

Intended Use

The Symansis MKA ELISA product range provides a quick, easy, more sensitive, and qualitatively better alternative to western blotting for kinase assay readouts, and it is designed to demonstrate the qualitative differences in the amounts of specific target proteins between samples.

Materials provided

Components	Quantity	Storage
Antibody-Coated Microwells. 2 x 8-microwell strips (note loss of vacuum on the plates/strips does affect their performance when stored as directed)	6 pouches	4°C, desiccated in the dark prevent multiple warm-ups and cool-downs
Biotin-Conjugated Detection Antibody. Contains 0.05% w/v Sodium Azide	6 x 1.7 ml	4°C in the dark prevent multiple warm-ups and cool-downs
Assay Control **NOTE:** Assay control is not analyte specific	1 x 1.4 ml	4°C in the dark prevent multiple warm-ups and cool-downs
Streptavidin Horseradish Peroxidase (SAV-HRP). Contains 3.3mM Thymol (KEEP PROTECTED FROM LIGHT)	11 ml	4°C in the dark prevent multiple warm-ups and cool-downs
Tetramethylbenzidine (TMB) Substrate (KEEP PROTECTED FROM LIGHT)	11 ml	4°C in the dark prevent multiple warm-ups and cool-downs
20 x Wash Buffer Concentrate. Contains 3.3mM Thymol	25 ml	4°C in the dark prevent multiple warm-ups and cool-downs
5 x Sample Dilution Buffer Concentrate. Contains 0.05% w/v Sodium Azide	25 ml	4°C in the dark prevent multiple warm-ups and cool-downs
Stop Solution	5.5 ml	4°C in the dark prevent multiple warm-ups and cool-downs
Plate Cover	3 sheets	Store RT, dry
Microwell Strip Holder	1	Store RT, dry

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Target Protein	Detection Antibody: Cap Colour	Microwell : Rim Colour
GSK3α (pS21)	Pink	Pink
GSK3β(pS9)	Blue	Blue

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Notes

1. **Sodium Azide** reacts with lead and copper plumbing to form explosive metals azides. Upon disposal, flush drains with copious amount of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water.

Additional Material Required (Not Supplied)

- Microtitre plate reader capable of measurements at or near 450nm
- Calibrated pipettes with disposable pipette tips. A manifold multichannel pipette is useful for large assays and to minimize pipetting errors.
- Cell Lysis Buffer Preparation for the recommended buffer formulation (Available as **cat # CLB001)**.
- Wash bottle, manual dispenser or automated plate washer
- Lint-free tissue
- Absorbent paper tissue
- Microcentrifuge Tubes
- Distilled or de-ionised Water

Procedural Notes

- Ensure all kit components are refrigerated when not in use. All reagents should be allowed to equilibrate to room temperature before use.
- Antibody-Coated microwell strips should be allowed to equilibrate to room temperature before opening the pouch. Any unused strips can be sealed and stored in the provided pouch (containing desiccant) at 4^{o}C for 3 months.
- Cell Lysates samples must be prepared using the **denaturing** cell lysis buffer containing **6M Urea** and protease and phosphatase Inhibitors for optimal assay performance **(see Cell Lysis Buffer Preparation).**
- Samples should be frozen if not analyzed. Avoid multiple freeze/thaw cycles of frozen samples. Samples must be allowed to thaw on ice prior to analysis.
- Cell lysate samples must be diluted to the desired test concentration with 1x diluted Sample Dilution Buffer **(see Reagent Preparation).**
- It is recommended that all test samples be run in duplicate.
- Total protein concentrations should be measured prior to running the assay using a urea compatible protein concentration kit.
- When pipetting reagents, maintain a consistent order of addition well to well. This ensures equal incubation times for all wells.
- Cover or cap all reagents when not in use.
- Do not mix reagents from other kits.
- Develop the color reaction in the dark at room temperature (~25°C).

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- Read Absorbances at 450 nm when there is a noticeable difference in color between the highest and lowest diluted samples or at least within 30 minutes of assay completion at room temperature.
- All residual wash liquid must be drained from the wells by decanting or aspiration followed by forceful tapping of the plate on absorbent paper tissue. Never insert absorbent paper into the wells. Ensure there is no residual wash buffer in the wells. Any remaining wash buffer may cause variation in assay results **(see Directions for Washing).**
- The TMB substrate is light-sensitive, avoid prolonged exposure to light. Avoid contact of TMB with metal, or colour may develop.

Safety Precautions

- TMB is toxic if inhaled or swallowed. Avoid contact with skin. Keep container tightly closed when not in use.
- Stop Solution is an acidic solution. Appropriate personal protection equipment must be worn (laboratory coat, gloves, eye protection).

Directions for Washing

- Incomplete washing will adversely affect the test outcome. All washing must be performed with wash buffer provided.
- Completely aspirate the liquid from all wells by gently lowering an aspiration tip into the bottom of the well. Care must be taken not to scratch the inside of the well.
- After aspiration, fill the wells with 0.3 ml of 1 x Wash Buffer **(see Reagent Preparation)**. Let soak for 15 to 30 seconds, and then aspirate the liquid. Repeat as directed in the **Assay Procedure**. After washing, invert the plate and forcibly tap on absorbent paper tissue.
- If a wash bottle is used, flood the plate with wash buffer, completely filling all the wells. After washing, to remove residual solution, invert the plate and forcibly tap on absorbent paper tissue. Do not allow the wells to dry at any time.
- Clean the underside of the wells with lint-free tissue.
- If an automated plate washer is used, follow the operational procedure and programme for 30 second soak cycles.

Reagent Preparation

- **1x Wash Buffer** : Allow 20x Wash Buffer Concentrate to equilibrate to room temperature. Mix to ensure any precipitated salt have re-dissolved. Stock solution can be warmed to 37°C to a facilitate the redissolving of any precipitated salt. Dilute 25 ml 20x Wash Buffer Concentrate into de-ionised or distilled water to a total volume of 500 ml. The diluted Wash Buffer is stable at 4^{o}C for up to 14 days. Store diluted wash buffer at 4°C between experiments. Washes should be performed with room temperature wash solutions.

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- **1x Sample Dilution Buffer :** Allow 5x Sample Dilution Buffer Concentrate to equilibrate to room temperature. Mix to ensure any precipitated salts have re-dissolved. Dilute 25 ml 5x Sample Dilution Buffer Concentrate into de-ionised or distilled water to a total volume of 125 ml. The diluted Sample Buffer is stable at 4^{o}C for up to 14 days.

Cell Lysis Buffer (denaturing conditions)

Denaturing Cell Lysis Buffer, containing **6M Urea must** be used for optimal assay performance. Symansis cannot guarantee optimal performance of the assay if 6M Urea is not included in the user's cell lysis buffer.

Suggested Denaturing Cell Lysis Buffer
(Available separately from Symansis, cat. # CLB001)

10 mM Tris, pH 7.4	150 mM NaCl
1 mM EDTA	1 mM EGTA
0.5% (v/v) Triton X®-100	**6M Urea**
2 μg/ml Leupeptin	10 μM Pepstatin
3 μg/ml Aprotonin	1 mM Sodium Orthovanadate (Na_3VO_4)
2 mM Sodium Pyrophosphate ($Na_4P_2O_7$)	5mM Sodium fluoride (NaF)

Sample Preparation

The following sample sizes are suggested as a guide for sample dilution. Dilute the samples in 1x Sample Dilution Buffer to produce a total of 100μl per well.

Recombinant protein	Cell lysates	Tissue lysates
1-20ng/well	20-60μg/well	100-200μg/well

Assay Procedure

The following procedure is for 12 x 8 microwell antibody coated strips. The researcher is advised to scale down or up appropriately, if processing less or more strips.

1. 2x8 antibody-coated microwell strips are individually packed in silver pouches. Equilibrate the microwell strips to room temperature as described in **Procedural Notes.**
2. Gently, place the strips in the microwell strip holder. Unused strips can be resealed and stored at 4^{o}C.
3. Add 100 μl of each diluted **cell lysate (sample)** and **Assay Control** into the appropriate well. 100 μl of 1x Sample Dilution Buffer alone should be added to **negative** control/blank wells.
    a. **Assay Control** allows the researcher to assess the performance of the assay reagents only. (**NOTE:** Assay control is not analyte specific as it is simply anti-sheep antibody)
4. Seal with plate cover and press firmly onto top of microwells. Incubate for 2 hours at room temperature.

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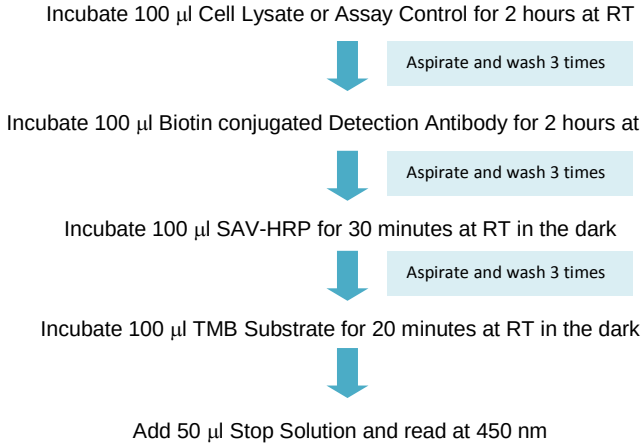
5. Gently remove the plate cover and wash wells. Thoroughly decant or aspirate solution from wells and discard the liquid. Wash wells 3 times with 0.3 ml of diluted 1x Wash Buffer **(see Directions for Washing)**.
6. Add 100 µl of the Biotin-Conjugated Detection Antibody to each well. Seal with plate cover and press firmly onto top of microwells. Incubate for 2 hours at room temperature.
7. Wash wells 3 times as directed in Step 5.
8. Add 100 µl SAV-HRP **(equilibrated to room temperature in the dark)** to each microwell. Seal with plate cover and press firmly onto top of microwells. Incubate for 30 minutes at room temperature protected from light .(SAV-HRP is light sensitive)
9. Wash wells 3 times as directed in Step 5.
10. Add 100 µl of TMB substrate solution **(equilibrated to room temperature).** Incubate the plate at room temperature in the dark. Check every 5 minutes and stop the reaction when a clear colour difference develops between untreated and treated samples (up to a maximum of 30 minutes)
    a. The MKA ELISA shows relative differences in amounts of phospho-proteins between samples. Colour development time will therefore vary between cell treatments and between different cell lines, tissues and recombinant proteins.
11. Add 50 µl of Stop Solution to each microwell. Gently tap the microtitre plate for a few seconds. The colour in each well should change from blue to yellow.
12. Wipe the underside of the microwells with lint free tissue and read absorbance at 450 nm **within 30 minutes** of stopping the reaction. Keep plate in the dark until read.

Assay Summary



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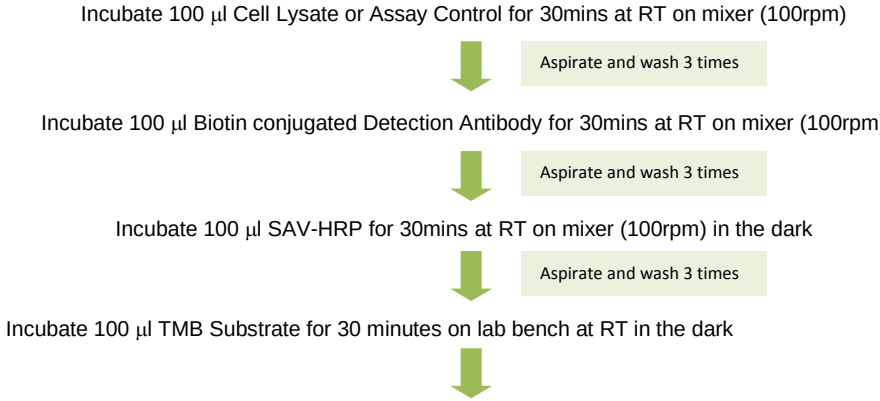
- Total Assay Time : Approximately 5 hours including washing

Optional Assay Procedure (fast protocol option)

Step 3 (addition of sample) and Step 6 (addition of detection antibody) incubation times can be shortened to **30 mins** each with the use of an orbital shaker. (100 rpm recommended)
Step 8, (addition of SAV-HRP) should be incubated on the orbital mixer for **30 mins**.
Step 10, (addition of TMB) should be incubated on a bench top for **30 mins**.

To minimize assay drift, it is essential to add samples and detection antibody in as short a time as is practical. This shortened time procedure may result in lower OD values compared to the standard procedure.

Optional Procedure Summary (fast protocol option)



- Total Assay Time : Approximately 2.5 hours including washing

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Troubleshooting

Problem	Possible Cause	Solution
High signal and background in all wells	Insufficient washing	Increase number of washes Increase time of soaking between washes
	Development time too long	Decrease the TMB incubation time before the stop solution is added
	Plate sealer or reservoir or pipette tips re-used, causing cross contamination	Use fresh plate sealer and reservoir and tips for each step
No signal	Reagent added in incorrect order, or incorrectly prepared	Review protocol
	Assay control has expired (If there is a signal in the sample wells)	Check the condition and expiry date of stored assay control
	Reagents were not at appropriate temperature	Allow reagents to come to room temperature (20-30°C) before performing assay
No signal when a signal is expected	Sample matrix is masking detection	More diluted sample recommended
	Samples contain insufficient protein target	More concentrated sample recommended
Unexpected variation in signal	Air bubbles formed in well after stop solution added	Check for and remove air bubbles before reading the plate
	Plate sealer or reservoir or pipette tips re-used, causing cross contamination	Use fresh plate sealer and reservoir and tips for each step
Edge effect	Uneven temperature around work surface	Avoid incubating plate in areas where environmental conditions vary Use plate sealer

Please visit our website
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for Symansis Multi-Kinase ELISA Companion Products

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