Sulfatase Activity Assay Kit

ARG83565 Sulfatase Activity Assay Kit can be used to measure Sulfatase in Tissue extracts, Cell lysate, Cell culture media, Other biological fluids

Catalog number: ARG83565

Package: 100 tests

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PRINCIPLE OF THE ASSAY

ARG83565 Sulfatase Activity Assay Kit provides a simple and sensitive method for monitoring sulfatase activity in various samples. The kit measures the hydrolysis of a sulfate ester to 4-nitrocatechol, which can be measured at a colorimetric readout at 515 nm.

MATERIALS PROVIDED & STORAGE INFORMATION

Store Positive Control and Substrate at-20 °C and protect from light, all other component at 2-8°C. Use the kit before expiration date.

Component	Quantity	Storage
Microplate	1 X 96-well plate	
Standard	1 vial (lyophilized)	4 °C
Positive Control	1 vial (lyophilized)	-20 °C
Assay Buffer	4x 30 ml	4 °C
Reaction Buffer	10 ml	4 °C
Substrate	1 vial (lyophilized)	-20 °C
Stop Solution	10 ml	4 °C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes and pipette tips
- Deionized or distilled water

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store Positive Control and Substrate at -20 °C and protect from light, all other component at 2-8°C. Use the kit before expiration date.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

Cell and bacteria- Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5 × 10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 10,000g 4 °C for 20 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

Tissue- Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 10,000g 4 °C for 20 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

REAGENT PREPARATION

- **Substrate:** Reconstitute the **Substrate** with **1 ml** of Reaction Buffer. Allow the **Substrate** keep on bench for few minutes. Make sure the **Substrate** is dissolved completely and mixed thoroughly before use.
  The diluted **Substrate** is stable for 4 weeks at-20°C.

- **Standard:** Reconstitute the **Standard** with 1 ml of distilled water. Allow the **Standard** keep on bench for few minutes. Make sure the **Standard** is dissolved completely. Perform 2-fold serial dilutions of the top standards to make the standard curve.
  The diluted **Standard** is stable for 4 weeks at-20°C.

- **Dye Reagent A:** Reconstitute the **Dye Reagent A** with **1 ml** of distilled water. Allow the **Dye Reagent A** keep on bench for few minutes. Make sure the **Dye Reagent A** is dissolved completely and mixed thoroughly before use.
  The diluted **Dye Reagent A** is stable for 4 weeks at-20°C.

ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

1. Add **80 µl** Reaction Buffer into all wells.
2. Add **10 µl** Sample into Sample wells
3. Add **10 µl** Distilled water into Control, Standard and Blank wells.
4. Add **10 µl** Positive Control into Positive Control wells.
5. Add **10 µl** Substrate into all wells.
6. Add **10 µl** Standard into Standard wells.
7. Mix well incubate for **30 min** at **37 °C**
8. Add **10 µl** per Dye Reagent into all wells.
9. Mix well. Read the OD at **515nm**.

Summary of Sulfatase Activity Assay Kit Procedure

Reagent	Sample	Control	Standard	Blank	Positive Control
Reaction Buffer	80 µl	80 µl	80 µl	80 µl	80 µl
Sample	10 µl	-	-	-	-
Distilled water	-	10 µl	10 µl	10 µl	-
Positive Control	-	-	-	-	10 µl
Substrate	10 µl	10 µl	10 µl	10 µl	10 µl
Standard	-	-	10 µl	-	-
Mix well incubate for 30 min at 37 °C					
Dye Reagent	100 µl	100 µl	100 µl	100 µl	100 µl
Read the OD at 515 nm					

CALCULATION OF RESULTS

Calculate the average absorbance values for each set of samples, standard and blank.

a.) Definition:

One unit of sulfatase activity is defined as the enzyme which generates 1 µmol of 4-nitrocatechol per minute at 37°C.

$C_{Protein}$: the protein concentration, mg/ml;

$C_{Standard}$: the concentration of Standard, 5 mmol/L = 5 µmol/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, N × 104;

$V_{Standard}$: the volume of standard, 0.01 ml;

V_{Sample}: the volume of sample, 0.01 ml;

V_{Assay}: the volume of Assay buffer, 1 ml;

T: the reaction time, 30 min.

b.) Calculation:

Formula:

a). According to the Protein Concentration:

Sulfatase (U/mg) = $(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / [(OD_{Standard} - OD_{Blank}) \times (C_{Protein} \times V_{Sample}) \times T]$

$=0.167 \times (OD_{Sample} - OD_{Control}) / [(OD_{Standard} - OD_{Blank}) \times C_{Protein}]$

b). According to the weigh:

Sulfatase (U/mg) = ($C_{Standard}$ x $V_{Standard}$) x (OD_{Sample}- $OD_{Control}$) / [($OD_{Standard}$- OD_{Blank}) x (V_{Sample} x W/ V_{Assay}) x T]

=0.167 × (OD_{Sample}- $OD_{Control}$) / [($OD_{Standard}$- OD_{Blank}) x W]

b). According to the Cells or bacteria:

Sulfatase ($U/10^4$) = ($C_{Standard}$ x $V_{Standard}$) x (OD_{Sample}- $OD_{Control}$) / [($OD_{Standard}$- OD_{Blank}) x (V_{Sample} x N/ V_{Assay}) x T]

=0.167 × (OD_{Sample}- $OD_{Control}$) / [($OD_{Standard}$- OD_{Blank}) x N]