

## ALT Activity Assay protocol.v1

A modified protocol for quantification of ALT activity in serum or plasma according to SIGMA ALDRICH® ALT Activity Assay Kit (MAK052) Product Information

### Procedures

1. All samples and standards should be run **in duplicates**.
2. Prepare Pyruvate Standards by **diluting 10 µl of Pyruvate Standard (100 nmole/µl) with 990 µl of ALT Assay Buffer** (final concentration of 1 nmole/µl).
3. **Add 0, 2, 4, 6, 8, and 10 µl into a 96 well plate**, generating 0 (blank), 2, 4, 6, 8, and 10 nmole/well standards. Add 20,18,16,14,12,10 µl of ALT Assay Buffer, respectively to bring the volume to 20 µl.
4. **Add 1–20 µl of samples** (in case of serum or plasma, add 2 µl or more) into wells of a 96 well plate. Bring samples to a final volume of 20 µl with ALT Assay Buffer. For unknown samples, it is suggested to test several volumes to make sure the readings are within the linear range with a reasonable incubation time (~30 min).
5. For the positive control (optional), **add 5 µl of the ALT Positive Control to wells**. Adjust well volume to 20 µl with ALT Assay Buffer.
6. **Set up the Master Reaction Mix according to the scheme in Table 1**. Prepare enough for the number of samples, positive controls, and standards.

Table 1. Master Reaction Mix (composition for 1 well)

Reagent	Volume
ALT Assay Buffer	86 µL
Fluorescent Peroxidase Substrate	2 µL
ALT Enzyme Mix	2 µL
ALT Substrate	10 µL

7. Add **100 µl of the Master Reaction Mix** to each of the standard, positive control, and test wells. Mix well using a horizontal shaker or by pipetting.
8. After 2–3 minutes, **take the initial measurement (T<sub>initial</sub>)**. For colorimetric assays, measure the absorbance at 570 nm (A<sub>570</sub>).
9. **Incubate the plate at 37 °C** taking measurements every 5 minutes. Protect the plate from light during the incubation.
10. Continue taking measurements **until the value of the most active sample is greater than the value of the highest standard**. At this time the most active sample is near or exceeds the end of the linear range of the standard curve.
11. **The final measurement** for calculating the enzyme activity would be the value before the most active sample is near or exceeds the end of the linear range of the standard curve.
12. Calculate the change in measurement from T<sub>initial</sub> to T<sub>final</sub>.

$$\Delta A_{570} = (A_{570})_{\text{final}} - (A_{570})_{\text{initial}}$$

Note: It is essential the initial and final measurements fall within the linear range of the reaction.

### Calculations

1. Correct for the background by subtracting the value obtained for the 0 (blank) standard from all readings. Plot the pyruvate standard curves.
2. Compare the  $\Delta$ measurement value ( $\Delta A_{570}$ ) of each sample to the standard curve to determine the amount of pyruvate generated between  $T_{\text{initial}}$  and  $T_{\text{final}}$ (B).
3. The ALT activity of a sample may be determined by the following equation:

$$\text{ALT Activity} = [B \times \text{Sample Dilution Factor}] / [(T_{\text{final}} - T_{\text{initial}}) \times V]$$

B = Amount (nmole) of pyruvate generated between  $T_{\text{initial}}$  and  $T_{\text{final}}$

$T_{\text{initial}}$  = Time of first reading in minutes.

$T_{\text{final}}$  = Time of penultimate reading in minutes.

V = sample volume (ml) added to well (note it is the diluted sample volume if so).

ALT activity reported as nmole/min/ml = mU/ml, where one milliunit (mU) of ALT is defined as the amount of enzyme that generates 1.0  $\mu$ mole of pyruvate per minute at 37 °C.

### Reagent preparation:

1. Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents.
2. Bring ALT Assay Buffer to room temperature before use.
3. Allow Fluorescent Peroxidase Substrate to come to room temperature before use. Mix well by pipetting, then aliquot and store, protected from light and moisture, at -20 °C.
4. Reconstitute ALT Enzyme Mix in 220  $\mu$ l of water. Mix well by pipetting, then aliquot and store at -20 °C. Use within two months of reconstitution.
5. Reconstitute ALT Substrate in 1.1 mL of ALT Assay Buffer. Mix well by pipetting, then aliquot and store at -20 °C. Keep cold while in use. Use within two months of reconstitution.
6. Reconstitute ALT Positive Control – Reconstitute in 100  $\mu$ L of water. Mix well by pipetting, then aliquot and store at -20 °C. Keep cold while in use. Use within two months of reconstitution.
- 7.