



- Standard Curve Preparation:** Add 10 μl of 100 mM Glycerol Standard to 990 μl of Glycerol Assay Buffer to generate 1 mM Glycerol Standard and mix well. Add 0, 2, 4, 6, 8, & 10 μl of 1 mM Glycerol Standard into series of wells in 96-well plate to generate 0, 2, 4, 6, 8, & 10 nmol/well of Glycerol Standards. Adjust the volume to 50 μl per well with Glycerol Assay Buffer.
- Reaction Mix:** Mix enough reagents for the number of assays (samples and Standards) to be performed. For each well, prepare 50 μl Reaction Mix containing:

	Reaction Mix
Glycerol Assay Buffer	46 μl
Glycerol Probe	2 μl
Glycerol Enzyme Mix	2 μl

Mix well. Add 50 μl of Reaction Mix to each well containing Standard and test samples. Mix.

- Measurement:** Incubate the plate at room temperature for 30 min. protected from light. Measure absorbance (OD 570 nm) in a microtiter plate reader. The reaction is stable for at least 2 hr.
- Calculation:** Subtract 0 Standard reading from all readings. Plot the Standard Curve. Apply the corrected sample reading to the Standard Curve to get B nmol of Glycerol amount in the sample wells.

Sample Glycerol Concentration: $C = B \times T/S = \text{nmol/well}$

Where: **B** = amount of glycerol from Standard Curve (nmol).
T = total volume of the sample (μl).
S = sample volume added into the reaction well (μl).

Glycerol molecular weight: 92.09 g/mol.

Glycerol can be expressed in nmol or nmol/well; alternatively as nmol/μg protein or nmol/μg lipid.

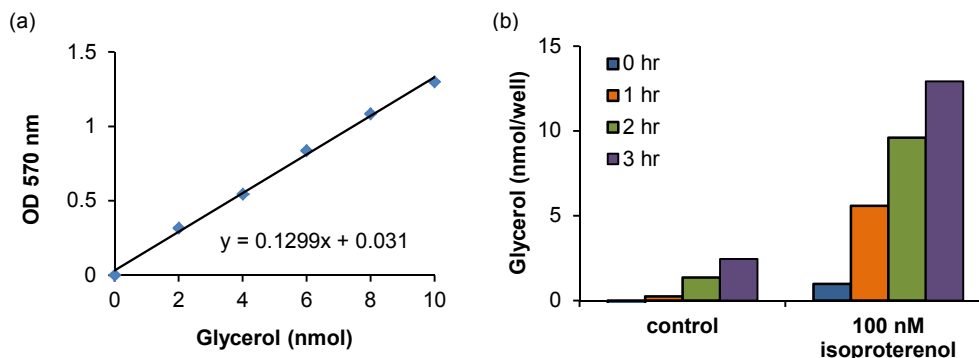


Figure: (a) Glycerol Standard Curve, (b) Measurement of glycerol level in media (50 μl) of 3T3-L1 cells treated with vehicle control (H₂O) or 100 nM Isoproterenol for 0-3 hr.

IX. RELATED PRODUCTS:

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| 3T3-L1 Lipolysis Fluorometric Assay Kit (K578) | Glucose Colorimetric/Fluorometric Assay Kit (K616) |
| 3T3-L1 Differentiation Kit (K579) | Insulin (human) ELISA Kit (K4742) |
| Adipogenesis Colorimetric/Fluorometric Kit (K610) | Adiponectin (human) Elisa Assay Kit (K4901) |
| Free Glycerol Colorimetric/Fluorometric Assay Kit (K630) | Adiponectin (mouse) Elisa Assay Kit (K4902) |
| Free Glycerol Colorimetric Assay Kit II (K634) | Adiponectin (rat) Elisa Assay Kit (K4903) |
| PicoProbe™ Free Glycerol Fluorometric Assay Kit (K643) | Resistin (human/mouse/rat) EIA Kit (K4767) |
| Glucose Uptake Colorimetric Assay Kit (K676) | Leptin (human) ELISA Kit (K4777) |
| Glucose Uptake Fluorometric Assay Kit (K666) | Triglyceride Quantification Colorimetric/Fluorometric Assay Kit (K622) |
| Cell Lysis Buffer (1067) | BCA Protein Quantitation Kit (K812) |

FOR RESEARCH USE ONLY! Not to be used on humans.