

BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

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CD38 Assay kit (Cat #: E-158)

COMPONENTS: CD38 Assay Solution: 15 ml, store at -80°C (for 100 wells; **avoid light exposure**)
CD38 Substrate: 3 ml, store at -80°C (**avoid light exposure**)
Reagent LA (500x): 25 µl, store at -80°C
Reagent LD (500x): 25 µl, store at 4°C (**briefly vortex vial prior to pipetting; do not freeze solution**)
10x Cell Lysis Solution: 25 ml, store at room temperature (**agitate vial to redissolve contents**)

PRODUCT DESCRIPTION: The CD38 assay kit measures the NADase activity and is based on detection of the tetrazolium dye formazan ($\epsilon = 18 \text{ mM}^{-1}\text{cm}^{-1}$ at 492 nm). The kit is suitable for measurement of CD38 activity present in cell/tissue lysate and serum samples. Reagents are stable for at least 1 year if stored and handled properly.

Preparation of cell/tissue extracts:

1. Bring up $\sim 10^5$ washed cells in 50 µl ice-cold 1x Cell Lysis Solution by pipetting up and down. Leave lysate on ice for 5 min with agitation. Tissue is homogenized in ice-cold 1x Cell Lysis Solution ($\sim 5 \text{ mg}$ tissue in 0.5 ml).
2. Centrifuge lysate in a cold microfuge at $\sim 14,000 \text{ rpm}$ for 5 min. Supernatant is harvested and stored at -80°C.
3. Use the BCA protein assay method to determine sample protein concentration. A suggested sample protein concentration range is 0.2 – 2 mg/ml. **Note:** Serum samples are diluted with dH₂O 3-fold prior to assay.

Preparation of working solutions:

Thaw CD38 Assay Solution, CD38 Substrate, and Reagent LA. *Do not over thaw.* Keep thawed solutions on ice shielded from light. CD38 Assay Solution is used to prepare solution LA, solution LD, and solution LAD. Each sample requires 50 µl of solution LA, LD, and LAD. See below for preparation of 0.5 ml each. The preparation can be scaled up proportionally.

Solution LA: Add 1 µl Reagent LA to 0.5 ml CD38 Assay Solution followed by mixing.

Solution LD: Briefly vortex Reagent LD vial. Add 1 µl to 0.5 ml CD38 Assay Solution followed by mixing.

Solution LAD: Add 1 µl Reagent LA and 1 µl Reagent LD to 0.5 ml CD38 Assay Solution followed by mixing.

Enzyme Assay

1. Use a plain (uncoated) 96-well plate. First set up 3 control wells designated as LA-C, LD-C, and LAD-C. Then set up 3 sample wells for each sample designated as LA-S, LD-S, and LAD-S.
2. Add 10 µl CD38 Substrate to each well set up above.
3. Add 20 µl 1x Cell Lysis Solution (as control for cell/tissue samples) or dH₂O (as control for serum samples) to each of the 3 control wells. Add 20 µl sample to each of the 3 sample wells. Gently agitate plate to mix contents.
4. Cover plate and incubate in a humidified 37°C incubator for 1 hour. *Do not use CO₂ incubator.*
5. Remove plate from incubator. Keep plate on bench (room temperature) shielded from light.
6. Proceed to prepare enough solution LA, solution LD, and solution LAD as described above. Add 50 µl solution LA to each LA-C and LA-S well. Add 50 µl solution LD to each LD-C and LD-S well. Add 50 µl solution LAD to each LAD-C and LAD-S well. Cover plate and incubate in a humidified 37°C incubator for 30 min.
7. Measure absorbance (O.D.) at 492 nm using a plate reader.
8. Subtract the O.D. of LA-C well and the O.D. of LD-C well from the O.D. of LAD-C well to generate **calibrated O.D.** for control (**calibrated O.D._{control}**).
9. Subtract the O.D. of LA-S well and the O.D. of LD-S well from the O.D. of LAD-S well to generate **calibrated O.D.** for each sample (**calibrated O.D._{sample}**).
10. Sample $\Delta\text{O.D.} = \text{calibrated O.D.}_{\text{control}} - \text{calibrated O.D.}_{\text{sample}}$.
11. Sample CD38 activity in IU/L = $\mu\text{mol}/(\text{L} \cdot \text{min}) = \Delta\text{O.D.} \times 1000 \times 80 \mu\text{l} / (90 \text{ min} \times 0.5 \text{ cm} \times 18 \times 20 \mu\text{l}) = \Delta\text{O.D.} \times 4.94$. CD38 activity may be normalized by sample protein concentration or GAPDH enzyme activity.

Additional Information:

- CD38 Assay Solution contains the DMSO and idonitrotetrazolium (INT). Please contact us or visit the product webpage for SDS information. 10x Cell Lysis Solution precipitates at cold temperature. Re-dissolve contents by agitation in warm water upon arrival.