Please cite this article as: Guilherme *et. al.*, (2018). Measuring CD38 Hydrolase and Cyclase Activities: 1,N^6-Ethenonicotinamide Adenine Dinucleotide (ε-NAD) and Nicotinamide Guanine Dinucleotide (NGD) Fluorescence-based Methods, *Bio-protocol* 8 (14): e2938. DOI: 10.21769/BioProtoc.2938.

bio-protocol www.bio-protocol.org/e2938 Vol 8, Iss 14, Jul 20, 2018
DOI:10.21769/BioProtoc.2938

Measuring CD38 Hydrolase and Cyclase Activities: 1,N^6-Ethenonicotinamide Adenine Dinucleotide (ε-NAD) and Nicotinamide Guanine Dinucleotide (NGD) Fluorescence-based Methods

Guilherme C. de Oliveira[1, #, *], Karina S. Kanamori[1, #], Maria Auxiliadora-Martins[2], Claudia C. S. Chini[1] and Eduardo N. Chini[1]

[1]Signal Transduction and Molecular Nutrition Laboratory, Kogod Aging Center, Department of Anesthesiology and Perioperative Medicine, Mayo Clinic College of Medicine, Rochester, USA; [2]Fundação de Apoio ao Ensino, Pesquisa e Assistência do Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo (FAEPA), São Paulo, Brazil

*For correspondence: oliveira.guilherme@mayo.edu

[#]Contributed equally to this work

[Abstract] CD38 is a multifunctional enzyme involved in calcium signaling and Nicotinamide Adenine Dinucleotide (NAD$^+$) metabolism. Through its major activity, the hydrolysis of NAD$^+$, CD38 helps maintain the appropriate levels of this molecule for all NAD$^+$-dependent metabolic processes to occur. Due to current advances and studies relating NAD$^+$ decline and the development of multiple age-related conditions and diseases, CD38 gained importance in both basic science and clinical settings. The discovery and development of strategies to modulate its function and, possibly, treat diseases and improve health span put CD38 under the spotlights. Therefore, a consistent and reliable method to measure its activity and explore its use in medicine is required. We describe here the methods how our group measures both the hydrolase and cyclase activity of CD38, utilizing a fluorescence-based enzymatic assay performed in a plate reader using 1,N^6-Ethenonicotinamide Adenine Dinucleotide (ε-NAD) and Nicotinamide Guanine Dinucleotide (NGD) as substrates, respectively.

Keywords: CD38, NAD$^+$, NADase, Cyclase, Hydrolase, NGD, ε-NAD, Aging

[Background] Current studies on age-related development of metabolic dysfunction and frailty are each day in more evidence. It is known that, as the aging progresses, the NAD$^+$ levels decrease in an expected process. Recent studies have shown that a reduction in nicotinamide adenine dinucleotide (NAD$^+$) is implicated in the development of age-associated metabolic decline (Massudi *et al.*, 2012). Increased NAD$^+$ levels *in vivo*, results in activation of pro-longevity and health span-related factors and improves several physiological and metabolic parameters of aging (Camacho-Pereira *et al.*, 2016), including muscle function, exercise capacity, glucose tolerance, and cardiac function in mouse models of natural and accelerated aging.

Due to its role in NAD$^+$ metabolism, the study of CD38 and its functions has been of great importance. CD38 was first identified in 1980 as a structural cell surface marker for the characterization of immune cells (Malavasi *et al.*, 2008; van de Donk *et al.*, 2016), and its first association as a NAD hydrolase enzyme was in the following decade (Kontani *et al.*, 1993). However, during the past years, its enzymatic

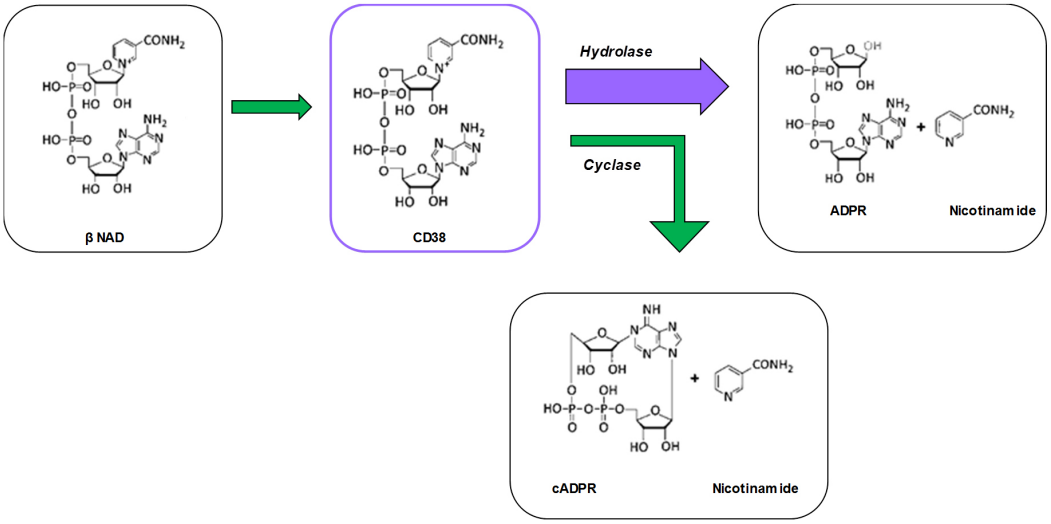
Please cite this article as: Guilherme *et. al.*, (2018). Measuring CD38 Hydrolase and Cyclase Activities: 1,N^6-Ethenonicotinamide Adenine Dinucleotide (ε-NAD) and Nicotinamide Guanine Dinucleotide (NGD) Fluorescence-based Methods, *Bio-protocol* 8 (14): e2938. DOI: 10.21769/BioProtoc.2938.

bio-protocol www.bio-protocol.org/e2938 Vol 8, Iss 14, Jul 20, 2018 DOI:10.21769/BioProtoc.2938

activities were more clearly elucidated. Initially, CD38 has been implicated to be responsible for the synthesis of the second messengers, cyclic ADP-ribose (cADPR), ADPR and nicotinic acid–adenine dinucleotide phosphate (NAADP) (Chini *et al.*, 2002). These products are involved in calcium signaling and control many biological processes including lymphocyte proliferation and insulin secretion (Kato *et al.*, 1999). However, its major enzymatic activity is the NAD^+ hydrolysis, placing CD38 as the major NADase in several mammalian tissues and as an important regulator of NAD^+-dependent processes (Aksoy *et al.*, 2006).

The primary catalytic reaction of CD38 involves the cleavage of the high energy β-glycosidic bond between nicotinamide and ribose. During catalysis, the removal of the nicotinamide from β-NAD is coupled with the formation of intermediates that are stabilized through H-bonds between their ribosyl groups and the catalytic residue Glu226, a residue required for the NADase and cyclase activity of the enzyme (Sauve *et al.*, 2000; Liu *et al.*, 2009). These intermediates are released from the catalytic site forming ADPR or cADPR (Figure 1). In general, the majority of the CD38 NADase catalytic activity will generate nicotinamide, but also ADPR and cADPR which have been shown to have second messenger signaling roles through the activation of ryanodine receptor (RYR2). The full description of these pathways and Ca^{2+} signaling can be found in the remarkable works of Galione (1994) and Chini and Dousa (1996). The roles of CD38 as a cyclase and of NAD-derived calcium messengers in physiology and pathology have been extensively reviewed (Sauve *et al.*, 2000; Chini, 2009).

Physiologically, CD38 has been implicated in the regulation of metabolism and the pathogenesis of the aging process, and of multiple conditions, such as obesity, diabetes, heart disease, asthma and inflammation. Therefore, the study of CD38, its activities, and possible modulators are of great interest. Our protocol presents a method of measuring its hydrolase and cyclase activities, utilizing ε-NAD and NGD techniques (Graeff *et al.*, 1994) with a fluorescence-based enzymatic assay performed in a plate reader, in a consistent and reproducible manner (Figure 2).



Please cite this article as: Guilherme *et. al.*, (2018). Measuring CD38 Hydrolase and Cyclase Activities: 1,N^6-Ethenonicotinamide Adenine Dinucleotide (ε-NAD) and Nicotinamide Guanine Dinucleotide (NGD) Fluorescence-based Methods, *Bio-protocol* 8 (14): e2938. DOI: 10.21769/BioProtoc.2938.

Figure 1. Schematic illustrating the reactions catalyzed by CD38 under physiological conditions

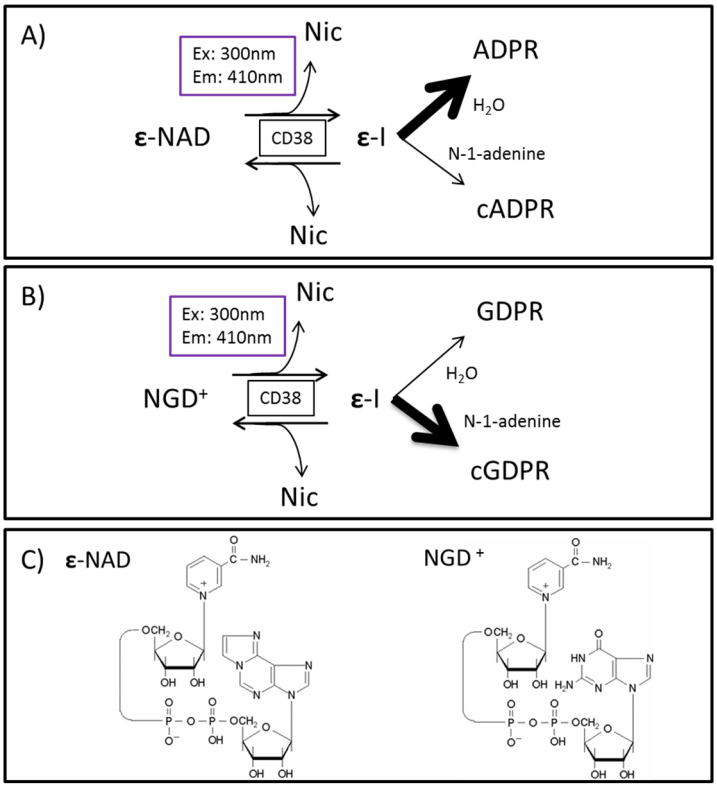


Figure 2. CD38 and substrate schematics. A. CD38 hydrolase activity (ε-NAD as substrate); B. Cyclase activity (NGD as substrate); Strong arrow indicates which product is preferentially formed in each reaction. C. Molecular structure of ε-NAD and NGD. *Nic = Nicotinamide, ε-I = enzyme-intermediate complex, (c)ADPR = (cyclic) ADP-ribose, (c)GDPR = (cyclic) GDP-ribose, Ex = excitation wavelength, Em = emission wavelength.

Materials and Reagents

1. Plastic tips 1,000 (Thermo Scientific®, Molecular Bioproducts, catalog number: 3101)
2. Plastic tips 200 (Thermo Fisher Scientific, Molecular Bioproducts, catalog number: 3551)
3. 96-well plate (Microfluor 1 White flat-bottom plate) (Thermo Fisher Scientific, Thermo Scientific™, catalog number: 7705)
4. 1.5 ml tubes
5. 60 mm culture dishes (Fisher Scientific, Fisherbrand™, catalog number: FB012921)
6. Tissues of interest: any tissue can be used to measure NAD^+/NADH levels
7. Cells of interest: we usually use A549, JURKAT, Patu 9888T to measure NAD^+/NADH levels
8. Bovine Serum Albumin (BSA) (Sigma-Aldrich, catalog number: A7906)
9. Sucrose (Sigma-Aldrich, catalog number: S0389-1KG)

Please cite this article as: Guilherme *et. al.*, (2018). Measuring CD38 Hydrolase and Cyclase Activities: 1,N^6-Ethenonicotinamide Adenine Dinucleotide (ε-NAD) and Nicotinamide Guanine Dinucleotide (NGD) Fluorescence-based Methods, *Bio-protocol* 8 (14): e2938. DOI: 10.21769/BioProtoc.2938.

10. Tris Base (Trizma® base, Sigma-Aldrich, catalog number: T6066)
11. Bio-Rad Protein Assay Dye Reagent Concentrate (Bio-Rad Laboratories, catalog number: 5000006)
12. CD38 human recombinant enzyme (R&D Systems, catalog number: 2404-AC-010)
13. CD38 inhibitor (Merck, Calbiochem, catalog number: 538763)
14. Anti-CD38 antibody–Isatuximab (Creative-Biolabs, catalog number: TAB-432CQ)
15. Nicotinamide guanine dinucleotide sodium salt (NGD) (Sigma-Aldrich, catalog number: N5131)
16. Nicotinamide 1, N6-ethenoadenine dinucleotide (ε-NAD) (Santa Cruz Biotechnology, catalog number: sc-215559)
17. MES (Sigma-Aldrich, catalog number: M3671)
18. Sodium chloride (Sigma-Aldrich, catalog number: S7653)
19. Nanopure water
20. HCl
21. Sucrose Buffer (see Recipes)
22. rhCD38 enzyme buffer (see Recipes)

Materials necessary to collect cells:

1. Scraper–Corning cell lifter (Corning, catalog number: 3008)
2. Trypsin-EDTA 0.25% (Thermo Fisher Scientific, Gibco™, catalog number: 25200056)
3. Phosphate Buffered Saline (PBS) 1x (Thermo Fisher Scientific, Gibco™, catalog number: 10010023)

Equipment

Note: The brands and models indicated are the ones used by our group, similar equipment can be used as well.

1. Pipettes (10, 200, 1,000 µl)
2. Scissors
3. Graduated cylinder
4. Repeat pipette (Eppendorf, model: Repeater® M4)
5. Scale (Mettler-Toledo International, model: AG104)
6. Microcentrifuge (Eppendorf, model: 5424)
7. Homogenizer (Tissue Tearor, Bio Spec Products, catalog number: 780CL-04)
8. Sonic Dismembrator (Fisher Scientific, model: Model 100 Sonic Dismembrator)
9. Spectrophotometer (BioTek Instruments, model: Epoch 2)
10. Vortex (Scientific Industries, model: Vortex-Genie 2, catalog number: G560)
11. Plate reader (Molecular Devices, model: SpectraMax Gemini XPS)

Software

Please cite this article as: Guilherme *et. al.*, (2018). Measuring CD38 Hydrolase and Cyclase Activities: 1,N^6-Ethenonicotinamide Adenine Dinucleotide (ε-NAD) and Nicotinamide Guanine Dinucleotide (NGD) Fluorescence-based Methods, *Bio-protocol* 8 (14): e2938. DOI: 10.21769/BioProtoc.2938.

1. Gen5 Microplate Reader and Imager Software (BioTek Instruments)
2. Microsoft Excel (Microsoft Corporation)
3. SoftMax Pro 6 (Molecular Devices, LLC)
4. GraphPad Prism 7 (GraphPad Software, Inc)

Procedure

The CD38 activity assay can be done mainly with three different sources of CD38, cells, tissues, and recombinant enzyme. We are going to describe the procedure for cells and tissues first, and then describe the procedure using recombinant CD38 enzyme. The assay is performed in a 96-well white opaque plate (refer to Notes for plate information), at least in duplicates, with a final volume of 200 µl per well.

A. Cell and tissue samples

It is necessary to lyse the cells and tissue samples to be able to measure CD38 activity. To do so, we lyse the samples in sucrose buffer with sonication. The optimal volume of buffer should be empirically determined for each cell type and pellet size, as well as tissue weight, to ensure efficient lysis and an optimal final concentration of proteins in the lysate. We normally use 300 µl for 20 mg of tissue, and 100 µl of sucrose buffer if using cells from a 60 mm dish (2-3 x 10^6 cells). Keep buffer and samples on ice during all the assay steps.

1. Sample preparation
   a. Cells: wash cells in PBS, collect them according to standard procedures suitable for each cell type (adherent cells via scraping or trypsinization; cells that grow in suspension via transferring and pelleting) in 1.5 ml tubes and pellet them by centrifugation (30 sec, at 11.7 *x g*). Aspirate supernatant, re-suspend cells with 100 µl of sucrose buffer, if using 2-3 x 10^6 cells) and sonicate the samples at 30-50 W of power and 20 kHz of frequency on ice, for 3 times of 5 sec each.
   b. Tissue: in a 2 ml tube, add the piece of tissue (approx. 20 mg), add 300 µl of sucrose buffer and homogenize with scissors and a mechanical homogenizer until there are no visible chunks. Then, sonicate as explained above for cells. Remember to keep samples on ice during this process to avoid that the heat generated degrades the CD38 enzyme.

      After sonicating, centrifuge samples for 10 min at 13.8 *x g*, and at 4 °C. Transfer supernatant to a new tube for protein measurement by the Bio-Rad protein assay, and following steps. Discard pellet.
2. Normalizing samples

   Normalize samples with sucrose buffer to obtain a mass of 20-100 µg of protein if tissue is the source of enzyme, or 50-100 µg of protein per well if cells are being used. The volume of sample to be pipetted into each well is 100 µl. For tissues with high CD38 expression, like spleen, aim to the lower end of the interval. Proceed to “Prepare reaction mix” step.

Please cite this article as: Guilherme *et. al.*, (2018). Measuring CD38 Hydrolase and Cyclase Activities: 1,N^6-Ethenonicotinamide Adenine Dinucleotide (ε-NAD) and Nicotinamide Guanine Dinucleotide (NGD) Fluorescence-based Methods, *Bio-protocol* 8 (14): e2938. DOI: 10.21769/BioProtoc.2938.

B. Recombinant CD38 enzyme

If using commercially available recombinant CD38 enzyme, the preparation of the assay differs from how it is done with cells and tissues. The total volume in the well is maintained at 200 µl, the reaction mix is the same (100 µl) but the volume of enzyme mix can vary if one wants to test an inhibitor or activator. This method can also be adapted to use with cells or tissue samples, although our group normally treat cells or animals previously with inhibitors/activators and proceed as described above.

1. Prepare test compounds/inhibitors

   Prepare a dilution of the test compounds 4x of the desired final concentration in sucrose buffer to a volume of 50 µl per well. Also, prepare a blank sample, with 50 µl of sucrose buffer and no test compounds. We recommend the use of a known CD38 inhibitor, such as 78c (50 nM final), or anti-CD38 antibodies as a control for the CD38 activity and test compounds. For human samples or human recombinant CD38 enzyme we suggest the use of Isatuximab (1 µg/ml), a well-known monoclonal anti-CD38 antibody already in Phase 3 clinical trials for Multiple Myeloma.

2. Prepare enzyme mix

   The enzyme mix, which contains Recombinant enzyme (10 ng/µl), BSA (40 mg/ml in water) and sucrose buffer, will have a total volume of 50 µl per well. We suggest an incubation period of 15 min at room temperature if antibodies are being used, based on our experience. For 78c no incubation is required, since this molecule only binds to the enzyme-substrate product. Calculate the number of wells that are going to be used and use the following proportion to prepare the enzyme mix: Recombinant enzyme 1 µl/well; BSA 4 µl/well; and sucrose buffer 45 µl/well.

Note: The following steps are common for both samples type.

3. Prepare reaction mix
   a. Based on which CD38 activity one wants to measure, two different substrates are added to sucrose buffer. For the hydrolase/NADase activity, ε-NAD is used. For the cyclase activity, NGD is the substrate of choice.
   b. Prepare the reaction mix based on the number of wells to be used, considering 100 µl of reaction mix per well. For each 1 milliliter of total reaction mix, 5 µl of 10 mM ε-NAD and 40 µl of 10 mM NGD should be added, in order to achieve a final concentration of 50 and 200 µM, respectively. Vortex the tube and leave it at room temperature.
4. Setting up the reaction
   a. Before start pipetting samples to the plate, make sure that the plate reader is properly configured. Set up machine to read fluorescence at 300 nm excitation and 410 nm emission. Configure settings of plate type (96-well opaque), analysis type (kinetics), time of analysis (at least 1 h, readings every 30 sec), define area to be read on plate, and set it up to shake once for 5 sec before start reading.

Please cite this article as: Guilherme *et. al.*, (2018). Measuring CD38 Hydrolase and Cyclase Activities: 1,N^6-Ethenonicotinamide Adenine Dinucleotide (ε-NAD) and Nicotinamide Guanine Dinucleotide (NGD) Fluorescence-based Methods, *Bio-protocol* 8 (14): e2938. DOI: 10.21769/BioProtoc.2938.

bio-protocol www.bio-protocol.org/e2938 Vol 8, Iss 14, Jul 20, 2018 DOI:10.21769/BioProtoc.2938

b. Finally, pipet 100 µl of normalized samples, or 50 µl of recombinant enzyme mix plus 50 µl of inhibitor/activator tested, at least in duplicates. It is advised to pipet sucrose buffer in a set of wells as a blank. Then, quickly add with a repeater pipette 100 µl of reaction mix to all wells, load plate on reader tray, and read.

Data analysis

Plot the values obtained in an X-Y graph. On the X-axis plot the time, and on the Y-axis plot the fluorescence unit values obtained during the reading process (Figure 3). For statistical analysis, we use GraphPad Prism. The enzymatic activity curves obtained can be compared to evaluate the effects of the compound studied in the CD38 enzyme. For instance, in Figure 3, the black curve "Buffer" indicates the enzyme function without a test compound (where sucrose buffer was added to the enzyme). The grey curve shows the effect of the CD38 inhibitor, 78c, clearly demonstrating that there was little increase in activity over time. The red and pink "Antibodies" curve shows the CD38 activity when an Anti-CD38 antibody, in two different concentrations, was added to the enzyme. The green curves represent two concentrations of an unknown test compound being evaluated for CD38 inhibition, in this case showing little effect on CD38 activity. "No enzyme" refers to wells where only sucrose buffer and reaction mix were added. Figures 3C and 3D show the raw data and graph of percentage of activity in a chosen time point for all the compounds previously described. To compare two data sets, we perform a Student's *t*-test.

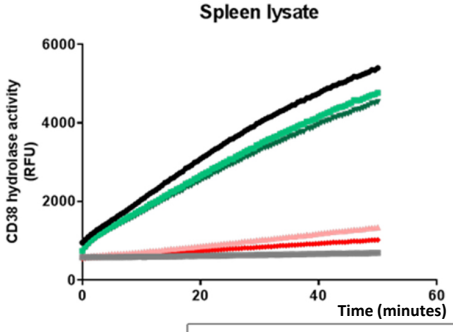
A

X	Group A		Group B		Group C		Group D		Group E		Group F	
X Title	Buffer		78c 200 nM		Anti-CD38 Ab - 1 µg/mL		Anti-CD38 Ab - 10 µg/mL		Test sample - 1 µg/mL		Test Sample - 10 µg/mL	
X	A:Y1	A:Y2	B:Y1	B:Y2	C:Y1	C:Y2	D:Y1	D:Y2	E:Y1	E:Y2	F:Y1	F:Y2
0.0	949.444	958.729	577.342	575.467	584.023	592.069	556.565	535.521	741.588	732.171	715.543	700.744
0.5	1027.919	1019.871	581.587	582.551	596.997	599.643	562.948	545.165	857.516	845.973	821.131	805.942
1.0	1099.634	1090.813	580.607	574.830	595.936	604.420	575.823	549.824	940.275	924.435	904.205	896.525
1.5	1165.595	1150.362	578.675	579.596	604.520	617.928	581.119	559.355	1018.852	1010.492	983.777	969.244
2.0	1220.328	1204.286	584.474	583.496	612.719	626.137	585.066	562.093	1079.080	1059.929	1043.048	1030.065
2.5	1287.138	1262.487	584.045	578.913	618.258	625.401	583.949	566.417	1142.160	1129.948	1102.266	1088.507
3.0	1334.373	1301.600	580.862	582.625	619.028	633.136	583.236	574.115	1184.632	1164.266	1144.878	1128.698
3.5	1395.857	1350.663	586.813	577.001	633.038	639.880	592.479	578.058	1228.690	1217.405	1191.922	1180.690
4.0	1443.636	1395.826	582.516	582.226	633.140	641.984	602.512	581.920	1279.994	1263.877	1238.234	1221.378
4.5	1490.684	1444.864	581.515	584.795	647							
5.0	1556.207	1501.894	581.424	581.769	654							
5.5	1595.079	1535.659	583.490	581.311	653							
6.0	1658.445	1597.007	589.041	581.779	659							
6.5	1713.728	1636.742	583.985	586.501	660							
7.0	1754.011	1681.229	583.533	583.986	665							

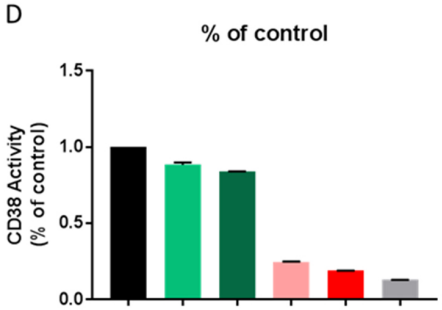
C

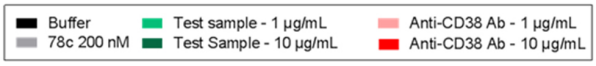
Group A	Group B	Group C	Group D	Group E	Group F
Buffer	78c 200 nM	Anti-CD38 Ab - 1 µg/mL	Anti-CD38 Ab - 10 µg/mL	Test sample - 1 µg/mL	Test Sample - 10 µg/mL
Y	Y	Y	Y	Y	Y
1	0.129397	0.247705	0.189467	0.898478	0.840115
1	0.125780	0.248785	0.187860	0.867062	0.839890

B



D





Please cite this article as: Guilherme *et. al.*, (2018). Measuring CD38 Hydrolase and Cyclase Activities: 1,N^6-Ethenonicotinamide Adenine Dinucleotide (ε-NAD) and Nicotinamide Guanine Dinucleotide (NGD) Fluorescence-based Methods, *Bio-protocol* 8 (14): e2938. DOI: 10.21769/BioProtoc.2938.

Figure 3. Data analysis on GraphPad Prism. A. Raw data; B. Final activity graph based on raw data analysis; C. Table of % of control values calculated per condition; D. Final % of control graph.

Notes

1. It is important that in key experiments, results are also confirmed by following the degradation of NAD^+, the natural substrate for both the CD38 NADase and cyclase enzymes, using HPLC to follow the hydrolysis of NAD^+ to ADPR and cADPR (Aksoy *et al.*, 2006).
2. NADase activity can also be verified by HPLC analysis, performed by anion-exchange chromatography using an AG MP-1 column (Bio-Rad) eluted with a non-linear gradient of trifluoroacetic acid, as described previously (Aksoy *et al.*, 2006). The nucleotides are detected by UV absorption at 254 nm, and the authenticity of NAD^+ is confirmed by co-elution with NAD^+ standards (Aksoy *et al.*, 2006).
3. We utilize 96-well standard opaque white plates for our assays, but we've obtained similar results with black plates, although they produce lower RFU values.

Recipes

1. Sucrose Buffer (500 ml)
   a. Weigh 42.7875 g of sucrose and 2.4228 g of Tris base to achieve a concentration of 0.25 M Sucrose and 40 mM Tris
   b. Add 400 ml of Nanopure water, add the sucrose and Tris, adjust pH to 7.4 with HCl and complete the volume to 500 ml in a graduated cylinder
   c. Store at 4 °C up to 6 months
2. rhCD38 enzyme buffer (5 ml)
   a. Weigh 24.4 mg of MES and 43.83 mg of NaCl to achieve a concentration of 25 mM MES and 150 mM NaCl
   b. Add 4 ml of Nanopure water, add the MES and NaCl, adjust pH to 6.5 with HCl and complete the volume to 5 ml in a graduated cylinder

Acknowledgments

This work was supported in part by grants from the Ted Nash Long Life Foundation, the Glenn Foundation for Medical Research via the Paul F. Glenn Laboratories for the Biology of Aging at the Mayo Clinic, a grant from Calico Laboratories, the Mayo Foundation, National Institutes of Health (NIH) grants from the National Institute of Aging (NIA, grant AG-26094), the Pancreatic Cancer SPORE project from NIH/NCI to E.N.C (grant CA102701-08), the Mayo Clinic Center for Cell Signaling in Gastroenterology (NIDDK P30DK084567). Maria Auxiliadora-Martins was supported by

a grant from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) Brazil (#2016/20089-4).

Declaration of interests: Dr. Chini holds a patent on the use of CD38 inhibitors for metabolic diseases.

References

1. Aksoy, P., White, T. A., Thompson, M. and Chini, E. N. (2006). Regulation of intracellular levels of NAD: a novel role for CD38. *Biochem Biophys Res Commun* 345(4): 1386-1392.
2. Camacho-Pereira, J., Tarrago, M. G., Chini, C. C. S., Nin, V., Escande, C., Warner, G. M., Puranik, A. S., Schoon, R. A., Reid, J. M., Galina, A. and Chini, E. N. (2016). CD38 dictates age-related NAD decline and mitochondrial dysfunction through an SIRT3-dependent mechanism. *Cell Metab* 23(6): 1127-1139.
3. Chini, E. N. (2009). CD38 as a regulator of cellular NAD: a novel potential pharmacological target for metabolic conditions. *Curr Pharm Des* 15(1): 57-63.
4. Chini, E. N., Chini, C. C., Kato, I., Takasawa, S. and Okamoto, H. (2002). CD38 is the major enzyme responsible for synthesis of nicotinic acid-adenine dinucleotide phosphate in mammalian tissues. *Biochem J* 362(Pt 1): 125-130.
5. Chini, E. N. and Dousa, T. P. (1996). Nicotinate-adenine dinucleotide phosphate-induced Ca^{2+}-release does not behave as a Ca^{2+}-induced Ca^{2+}-release system. *Biochem J* 316 (Pt 3): 709-711.
6. Galione, A. (1994). Cyclic ADP-ribose, the ADP-ribosyl cyclase pathway and calcium signalling. *Mol Cell Endocrinol* 98(2): 125-131.
7. Graeff, R. M., Walseth, T. F., Fryxell, K., Branton, W. D. and Lee, H. C. (1994). Enzymatic synthesis and characterizations of cyclic GDP-ribose. A procedure for distinguishing enzymes with ADP-ribosyl cyclase activity. *J Biol Chem* 269(48): 30260-30267.
8. Kato, I., Yamamoto, Y., Fujimura, M., Noguchi, N., Takasawa, S. and Okamoto, H. (1999). CD38 disruption impairs glucose-induced increases in cyclic ADP-ribose, $[Ca^{2+}]_i$, and insulin secretion. *J Biol Chem* 274(4): 1869-1872.
9. Kontani, K., Nishina, H., Ohoka, Y., Takahashi, K. and Katada, T. (1993). NAD glycohydrolase specifically induced by retinoic acid in human leukemic HL-60 cells. Identification of the NAD glycohydrolase as leukocyte cell surface antigen CD38. *J Biol Chem* 268(23): 16895-16898.
10. Liu, Q., Graeff, R., Kriksunov, I. A., Jiang, H., Zhang, B., Oppenheimer, N., Lin, H., Potter, B. V., Lee, H. C. and Hao, Q. (2009). Structural basis for enzymatic evolution from a dedicated ADP-ribosyl cyclase to a multifunctional NAD hydrolase. *J Biol Chem* 284(40): 27637-27645.
11. Malavasi, F., Deaglio, S., Funaro, A., Ferrero, E., Horenstein, A. L., Ortolan, E., Vaisitti, T. and Aydin, S. (2008). Evolution and function of the ADP ribosyl cyclase/CD38 gene family in physiology and pathology. *Physiol Rev* 88(3): 841-886.
12. Massudi, H., Grant, R., Braidy, N., Guest, J., Farnsworth, B. and Guillemin, G. J. (2012). Age-associated changes in oxidative stress and NAD^+ metabolism in human tissue. *PLoS One* 7(7):

e42357.

13. Sauve, A. A., Deng, H., Angeletti, R. H. and Schramm, V. L. (2000). A covalent intermediate in CD38 is responsible for ADP-ribosylation and cyclization reactions. *J Am Chem Soc* 122(33): 7855-7859.
14. van de Donk, N. W., Janmaat, M. L., Mutis, T., Lammerts van Bueren, J. J., Ahmadi, T., Sasser, A. K., Lokhorst, H. M. and Parren, P. W. (2016). Monoclonal antibodies targeting CD38 in hematological malignancies and beyond. *Immunol Rev* 270(1): 95-112.