LipidLaunch™ LNP Apparent pK_a Assay Kit (TNS Method)

Item No. 702680

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GENERAL INFORMATION

Materials Supplied

Item Number	Item Name	96 Well Quantity/Size	960 Well Quantity/Size	Storage Temperature
400634	TNS Probe Solution	1 vial/50 µl	1 vial/500 µl	-20°C
400635	TNS Buffer Solution pH 4.0	1 vial/1 ml	1 vial/10 ml	RT
400636	TNS Buffer Solution pH 4.5	1 vial/1 ml	1 vial/10 ml	RT
400637	TNS Buffer Solution pH 5.0	1 vial/1 ml	1 vial/10 ml	RT
400638	TNS Buffer Solution pH 5.5	1 vial/1 ml	1 vial/10 ml	RT
400639	TNS Buffer Solution pH 6.0	1 vial/1 ml	1 vial/10 ml	RT
400640	TNS Buffer Solution pH 6.4	1 vial/1 ml	1 vial/10 ml	RT
400641	TNS Buffer Solution pH 6.8	1 vial/1 ml	1 vial/10 ml	RT
400642	TNS Buffer Solution pH 7.2	1 vial/1 ml	1 vial/10 ml	RT
400643	TNS Buffer Solution pH 7.6	1 vial/1 ml	1 vial/10 ml	RT
400644	TNS Buffer Solution pH 8.0	1 vial/1 ml	1 vial/10 ml	RT
400645	TNS Buffer Solution pH 8.5	1 vial/1 ml	1 vial/10 ml	RT
400646	TNS Buffer Solution pH 9.0	1 vial/1 ml	1 vial/10 ml	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

This kit may not perform as described if any reagent or procedure is replaced or modified.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section (see page 3) and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader with the ability to measure fluorescence with excitation and emission wavelengths of 320 and 450 nm, respectively
2. Adjustable pipettes; multichannel or repeating pipettor recommended
3. An orbital microplate shaker
4. A source of pure water; glass-distilled water or deionized water is acceptable *NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000).*
5. Solid, black 96-well plates

INTRODUCTION

Background

The inclusion of ionizable lipids, lipids whose charge depends on the environment, in lipid nanoparticles (LNPs) is key for tuning the utility of LNPs for various purposes.[1] Therefore, the acid dissociation constant (pK_a) is an important parameter to consider in the development of LNP formulations for *in vitro* use or *in vivo* nucleic acid delivery.[1-4] The apparent pK_a of the LNP, the average ratio of all ionized to deionized groups comprising it, determines its ionization and surface charge, which affect how well the LNP is trafficked in the body and within the cell.[1,2] The stability, potency, and toxicity of LNPs also depend on the apparent pK_a.[1] For efficient trafficking and delivery of siRNA, mRNA, or RNA payloads, the optimal apparent pK_a for LNPs is typically 6-7 but varies depending on the route of administration, target organ, desired immune response, and cell type, among other factors.[1,2] The pK_a and surface charge of the LNP can be tuned by using different ionizable lipids, by combining ionizable lipids in different molar ratios, or by using selective organ targeting (SORT) components in the formulation.[1,3] For example, LNPs with a net negative charge are targeted to the spleen after intravenous injection while positively charged LNPs target the lung.[2,4] Therefore, consideration of the LNP pK_a is essential to the safety and efficacy of LNPs used *in vivo*.

About This Assay

Cayman's LipidLaunch™ LNP Apparent pK_a Assay Kit (TNS Method) provides a robust and easy-to-use platform for testing the apparent/global pK_a of formulated LNPs. The assay uses a fluorogenic probe, 6-(*p*-toluidino)-2-naphthalenesulfonic acid sodium salt (TNS). This probe fluoresces in association with positively charged membranes, which can be easily quantified using a fluorescence plate reader at excitation and emission wavelengths of 320 and 450 nm, respectively. TNS loses fluorescence as it dissociates, so a titration of the pH in the presence of LNPs results in a curve, with the 50% maximal fluorescence point called the apparent pK_a of the particle.

PRE-ASSAY PREPARATION

Sample Preparation

LNPs (dialyzed into a neutral buffer) should be diluted in the same neutral buffer such that the final lipid concentration is approximately 500 µM. The final required volume for duplicate samples is at least 150 µl. A final in-assay 20-fold dilution will result in a lipid concentration of approximately 25 µM without excessive assay buffer dilution.

For example: Producing LNPs using the LipidLaunch™ SM-102 LNP Exploration Kit (Item No. 35425) as directed in the kit booklet would result in a final lipid concentration of approximately 2.5 mM, which should be diluted about 5-fold in buffer to achieve the specified 500 µM concentration.

Reagent Preparation

1. **Buffer Set**

   For each plate of samples to be tested, mix 100 µl of each of the 12 TNS Buffer Solutions (Item No. 400635 through 400646) with 900 µl of room temperature (21-24°C) pure water to make 1 ml of each 1X TNS Buffer Solution (pH 4.0 through 9.0). Testing and recording the pH of each diluted solution is recommended because variables such as the temperature and type of water used can affect the final pH. These actual pH values will be used to graph the final results. Multichannel reservoirs can be very helpful for making dilutions and pipetting reagents into the final plate. Once thawed, the TNS Buffer Solutions may be stored at room temperature for at least six months.

2. **TNS Probe Solution**

   This vial contains 10 mM TNS Probe Solution (Item No. 400634) in DMSO. For each plate of samples to be tested, mix 8.3 µl of TNS Probe Solution with 992 µl of pure water to make an 83 µM working TNS probe solution. The diluted probe will be stable at room temperature for 1-2 hours. If all of the TNS Probe Solution will not be used at one time, store it at -20°C, where it will be stable for at least 12 months.

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. However, it is necessary to have the entire pH titration curve for each sample on a single plate. It is suggested that each sample be assayed in duplicate. It is suggested that the contents of each well be recorded on the template sheet provided on page 17. A typical layout of samples to be measured in duplicate is shown in Figure 1, below.

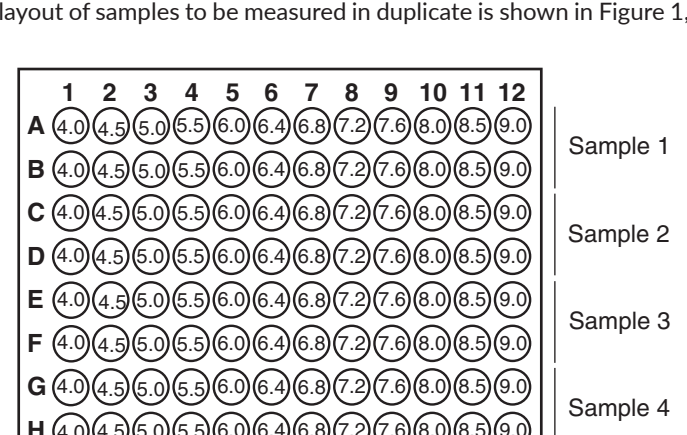


Figure 1. Sample plate format

Pipetting Hints

- It is recommended that a multichannel pipette be used to deliver reagents to the wells. This saves time and helps maintain more precise incubation times.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

General Information

- The final volume of the assay is 100 µl in all the wells.
- All reagents should be prepared as described above and kept at room temperature before beginning the assay.
- It is recommended to assay the samples in duplicate, but it is at the user's discretion to do so.
- The assay is performed at room temperature.
- Monitor the fluorescence with an excitation wavelength of 320 nm and an emission wavelength of 450 nm.

Performing the Assay

1. Starting with column 1 of a black 96-well plate, add 90 µl of 1X TNS Buffer Solution pH 4.0 to as many wells in the column as needed for the samples. Continue adding the remaining 1X pH gradient buffers through column 12. Refer to Figure 1, on page 10, for sample plate layout.
2. Add 5 µl of the first diluted LNP sample to each well across one row of the plate. Continue with the remaining samples in the other rows. Each sample must be added to all the wells in a row to properly determine the apparent pK_a of the sample. Duplicate sample loading is recommended.
3. Add 5 µl of the working TNS probe solution to each well for a final concentration of 4.15 µM TNS.
4. Incubate for 20 minutes at room temperature on an orbital shaker protected from light.
5. Read the plate with an excitation wavelength of 320 nm and an emission wavelength of 450 nm. It may be necessary to optimize the gain setting to allow for the measurement of all samples.

ANALYSIS

Calculations

1. Determine the average relative fluorescence (ARF) of each sample at each pH by subtracting the average fluorescence of the pH 9.0 wells from all other wells of that sample.
2. For each sample, graph the ARF against the corresponding pH measured in the **Pre-Assay Preparation** section (see page 8). Using a four-parameter regression, calculate the pH at 50% of the maximal fluorescence. This value is the apparent pK_a of the sample according to the TNS method.[2]

Performance Characteristics

Sample Data:

The data presented here is an example of data typically produced with this kit; however, your results will not be identical to these. Do not use the data below to directly compare to your samples. Your results could differ substantially.

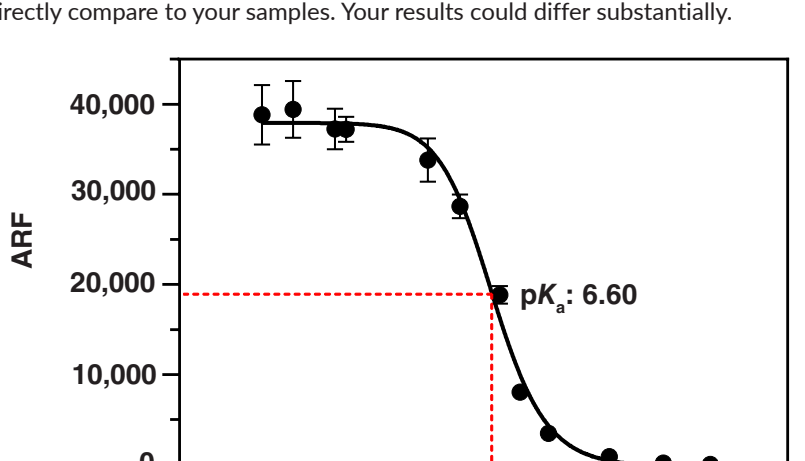


Figure 2. Apparent pK_a measurement of SM-102. Data are plotted as the mean ARF of triplicate measurements, with error bars representing the standard deviation. Four-parameter non-linear regression was used for the purpose of calculating the apparent pKa of 6.60 at 50% of the maximum ARF.

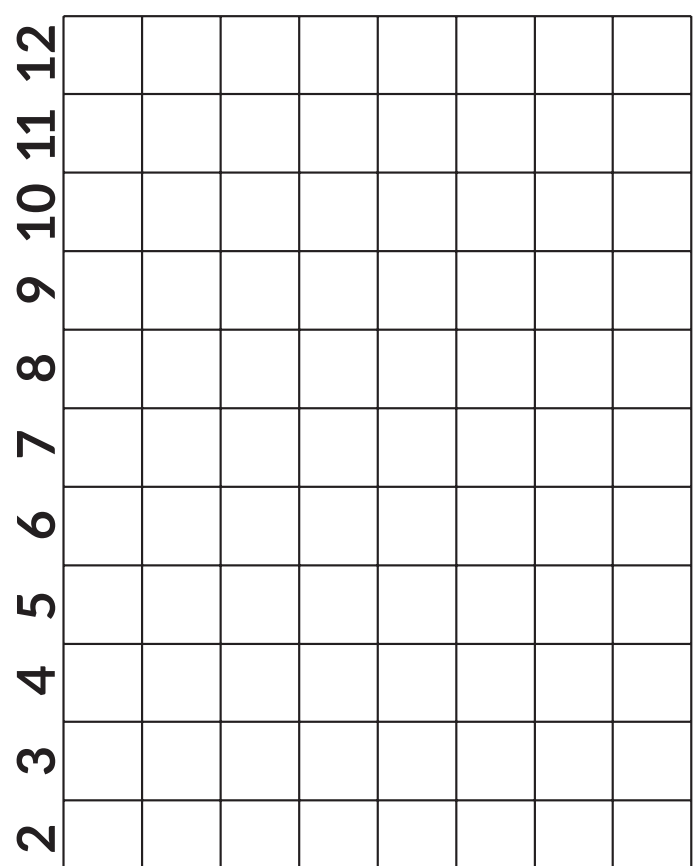
RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
RFUs are high across all pH values	Permanently charged lipid included in LNP	Cationic lipids are incompatible with this kit. Only test ionizable lipid-based particles.
RFUs are low across all pH values	A. LNPs are not stable or were not formed properly B. Ionizable lipid was not incorporated into LNP	A. Assess particle integrity/size using a different method, such as dynamic light scattering first B. Ensure the mixing method is valid and all reagents are of high quality
Incorrect/unexpected apparent pK_a	pH values of buffers are different from the target	A. Ensure all reagents are at room temperature (20-25°C) prior to beginning B. Test and record the pH of each diluted buffer and use these numbers to graph results

References

1. Patel, P., Ibrahim, N.M., and Cheng, K. The importance of apparent p*Ka* in the development of nanoparticles encapsulating siRNA and mRNA. *Trends Pharmacol. Sci.* **42(6)**, 448-460 (2021).
2. Carrasco, M.J., Alishetta, S., Alameh, M.-G., *et al.* Ionization and structural properties of mRNA lipid nanoparticles influence expression in intramuscular and intravascular administration. *Commun. Biol.* **4(1)**, 956 (2021).
3. Shobaki, N., Sato, Y., and Harashima, H. Mixing lipids to manipulate the ionization status of lipid nanoparticles for specific tissue targeting. *Int. J. Nanomedicine.* **13**, 8395-8410 (2018).
4. Cheng, Q., Wei, T., Farbiak, L., *et al.* Selective organ targeting (SORT) nanoparticles for tissue specific mRNA delivery and CRISPR-Cas gene editing. *Nat. Nanotechnol.* **15(4)**, 313-320 (2020).



NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.

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