

Background

The inclusion of ionizable lipids within lipid nanoparticles (LNPs) is essential for optimizing their function across different applications. Consequently, the acid dissociation constant (**pKa**) is a critical parameter in the design of LNP formulations for either *in vitro* use or *in vivo* nucleic acid delivery.

The **apparent pKa** of an LNP, defined as the average ratio of ionized to deionized groups that compose it, determines the degree of ionization and surface charge. These characteristics directly influence how efficiently the LNP is transported throughout the body and within cells. The **stability**, **potency**, and **toxicity** of LNPs also depend on this apparent pKa value.

For optimal delivery and trafficking of siRNA, mRNA, or other RNA payloads, the most effective apparent pKa typically falls between **6 and 7**, though it can vary according to factors such as the administration route, target organ, immune response desired, and cell type.

About this assay

The **NanOZ LNP Apparent pKa Assay Kit (TNS Method)** provides a reliable and ready-to-use platform for determining the apparent or global pKa of formulated LNPs.

The assay employs 6-(p-toluidino)-2-naphthalenesulfonic acid sodium salt (TNS) that fluoresces when bound to **positively charged membranes**. It can be quantified using a fluorescence plate reader with excitation and emission wavelengths of **320 nm** and **450 nm**, respectively.

As TNS dissociates, its fluorescence decreases. Therefore, titrating the pH in the presence of LNPs produces a fluorescence curve, and the pH corresponding to **50% of the maximal fluorescence** is defined as the **apparent pKa** of the nanoparticle.

Pre-Assay Preparation

1. Sample preparation

Dilute LNPs for a final lipid concentration of **~1 mM**. We recommend using the same pH-neutral buffer as that used for dialysis. The minimum volume required for duplicate measurements is **100 µl**.

2. TNS Probe Solution preparation

Working Probe Solution For each plate of samples to be analyzed, mix **20 µl** of the TNS Probe Solution (**5 mM** in DMSO) with **980 µl** of pure water to create a Working Probe Solution.

⚠ The diluted probe remains stable for **1–2 hours** at room temperature (RT). If not used immediately, store the stock solution at **-20°C**, where it will remain stable for at least **12 months**.

Assay Protocol

1. Plate set-up

Ensure that the **full pH titration range** is present on the same plate as the sample. It is recommended that each sample be analyzed **in duplicate (figure 1)**.

General Information

- The final reaction volume in each well is **100 µl**.
- All reagents should be prepared as described above and kept at **RT** before starting the assay.
- The assay should be carried out at **RT**.
- Measure fluorescence using an **excitation wavelength of 320 nm** and an **emission wavelength of 450 nm**.

2. Performing the assay

a. In a black 96-well plate, add **5 µl** of the diluted LNP sample to each well across one row. Duplicate loading is recommended

Repeat for additional samples in subsequent rows if needed.

b. Add **90 µl of 1X Buffer Solution pH 4.0** to the wells in column 1, add **90 µl of 1X Buffer Solution pH 4.5** to the wells in column 5, ...

Continue by adding the remaining 1X buffers sequentially across the plate (columns 3 - 12) to cover the full pH range of 4.0 - 9.0.

c. Add **5 µl** of the working TNS probe solution to each well.

d. Incubate the plate for **20 min** at RT on an orbital shaker, protected from light.

e. Read the fluorescence using an excitation wavelength of **320 nm** and an emission wavelength of **450 nm**.

Use, handling and storage

For Research Use Only. Not for use in humans. Not for use in diagnostic or therapeutic purposes.

Shipping conditions: Gel Pack.

Storage conditions: TNS -20°C, Buffer 4°C.

Shelf life: 1 year from the date of purchase.

Kit contents

PKA2000: 50 µL of 5mM TNS + 2 mL of each buffer (x12)

Certificate of analysis on demand.

Assay Protocol

Plate Set up

This analytical format is designed for the simultaneous evaluation of multiple samples (here, four) across a full range of pH conditions.

Although the general arrangement of wells is flexible, it is mandatory that each sample **is tested across the entire pH gradient, from 4.0 to 9.0**, to capture a complete titration curve.

This approach utilizes a duplicate measurement strategy (two adjacent rows per sample) to ensure data reliability and accuracy.

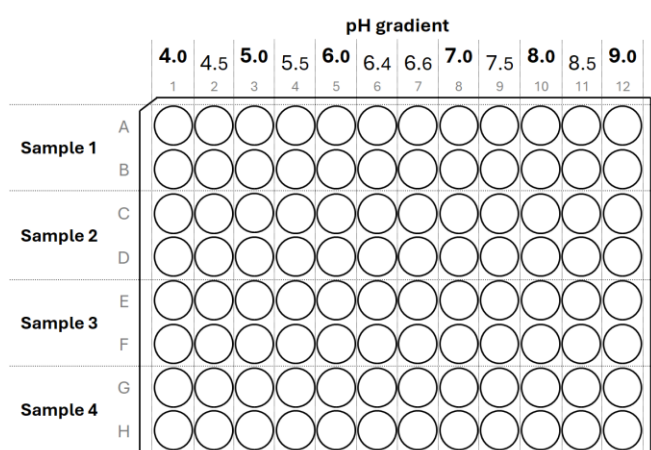


Figure 1. Plate set-up example.

Analysis

Calculations

a. For each sample, calculate the **Average Relative Fluorescence (ARF)** at every pH by subtracting the mean fluorescence of the **pH 9.0 wells** (background) from the fluorescence values of all other wells.

b. **Plot the ARF versus measured pH** (recorded during pre-assay preparation). **Using a four-parameter logistic regression**, determine the pH corresponding to 50% of the maximum fluorescence. This value represents the **apparent pKa** of the sample, as defined by the TNS method.

Performance characteristics

Sample Data. The results presented here serve as representative examples of data obtained using this kit.

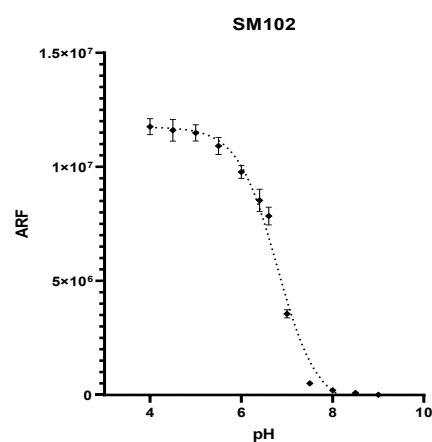


Figure 2. Apparent pKa measurement of SM-102. Means of 8 measurements in duplicate.

Troubleshooting

Problem	Possible cause	Recommended solutions
RFUs are high across all pH values	Presence of permanently charged lipids in the formulation	Cationic lipids are incompatible with this kit. Use only ionizable lipid-based nanoparticles.
RFUs are low across all pH values	(A) LNPs are unstable or improperly formed. (B) Ionizable lipid was not incorporated.	(A) Assess particle integrity or size using another analytical method, such as Dynamic Light Scattering (DLS). (B) Verify that the mixing protocol is valid and that all reagents are of high quality.
Incorrect or unexpected apparent pKa	Buffer pH values differ from target	(A) Ensure all reagents are equilibrated to 20–25°C before use. (B) Measure and record the pH of each diluted buffer and use those actual values for data analysis.

Purchaser Notification | Conditions of Sale

This product is sold in accordance with our general conditions of sale that you can find on our website: <https://ozbiosciences.com/content/3-terms-and-conditions>.