Product Number

926-20002 – Odyssey 28 KD Loading Indicator – 800 nm

926-20004 – Odyssey 28 KD Loading Indicator – 700 nm

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Odyssey® Loading Indicators

P/N	Description
926-20004	Odyssey 28kD Loading Indicator, 700nm, 2 x 1mL, sufficient for 400 lanes at 5µL per lane
926-20002	Odyssey 28kD Loading Indicator, 800nm, 2 x 1mL, sufficient for 400 lanes at 5µL per lane
Sample (926-20012, 926-20014)	Odyssey 28kD Loading Indicator, 700nm or 800 nm, 100µL, sufficient for 20 lanes at 5µL per lane

Specifications

Shelf Life: 6 months from date of receipt
Supplied in 4X Loading Buffer: 125mM Tris-HCl (pH 6.8), 50% (v/v) glycerol, 4% (w/v) SDS, and 0.2% (w/v) Orange G

NOTE: Crystals of SDS may form at temperatures lower than 28°C. Before use, warm buffer slightly while mixing gently until crystals disappear.

Product Description

Odyssey Loading Indicators are comprised of a single 28kDa recombinant protein labeled with a 700nm or 800nm fluorescent tag to allow direct visualization of the Loading Indicator on a Western blot. The Odyssey Loading Indicators are designed to provide a simple and convenient method for determining consistency of sample loading between gel lanes and Western blot transfer.

Applications

Housekeeping proteins (e.g. GAPDH, actin, tubulin) are routinely used as internal reference controls to normalize Western blot data. However, for accurate normalization, it is critical that the expression level of the housekeeping protein is unaffected by experimental conditions, and has a constant, stable expression level for all samples. The Odyssey Loading Indicator is not subject to up or down regulation as a function of sample treatment. Differences in signal between a housekeeping protein and the Loading Indicator can be used as part of the process to determine if expression of a housekeeping protein is influenced by sample treatment and therefore should be avoided for normalization experiments. For a detailed protocol, see the *Validating a Housekeeping Protein* protocol (licor.com/HKP-Validation).

For use, samples such as cell lysates are prepared at equal protein concentration and volume that includes the Odyssey Loading Indicator. After separation by SDS PAGE, proteins are transferred to immobilizing membranes, processed through standard Western blot procedures, and imaged on the Odyssey® Family of Imagers or imagers with similar spectral properties.

Procedure for Using the Odyssey Loading Indicators

Sample Dilution

1. Determine the Final Volume per well for all samples to load based on the well capacity of your gel (See Table 1 for examples).
2. Determine the Sample Volume required to add the proper amount of Odyssey Loading Indicator. Sample Volume should be equal to 75% of the Final Volume per well.
    a. For Example, if Final Volume per well is 10 µL, Sample Volume should be 7.5 µL
3. Dilute samples to equal concentration with appropriate sample buffer to equal the Sample Volume determined in step 2.

    Note: All samples should have the same final concentration and volume

Preparation of Odyssey Loading Indicator

1. Determine the volume of Odyssey Loading Indicator needed for all samples based on the Final Volume per well in step 1 (see Table 1 for examples). The amount of Odyssey Loading Indicator should be equal to 25% of the Final Volume per well for each sample.
    a. For Example, if Final Volume per well is 10 µL, Odyssey Loading Indicator should be 2.5 µL per sample
2. Prepare Odyssey Loading Indicator solution

    Note: Prepare only sufficient Loading Indicator with β-mercaptoethanol (β-ME) for the experiment; do not store.
    a. Determine the total amount of Odyssey Loading Indicator solution needed.
        i. Total Odyssey Loading Indicator= (Odyssey Loading Indicator X number of samples) X (1.20)*

        * 20% overage

        Example: (2.5 µL x 10 samples) x 1.20 = 30 µL
    b. Mix Odyssey Loading Indicator with β-ME at a ratio of 1µl β-ME for every 9µL of Odyssey Loading Indicator needed.
        i. Example: Add 3µl β-ME to 27µL of Odyssey Loading Indicator.

Combining Sample and Loading indicator

Add the prepared amount of Odyssey Loading Indicator to each sample.

Table 1. Volumes of Odyssey Loading Indicator to use for different sample amounts.

Sample Volume+ Buffer	Odyssey Loading Indicator (with β-ME)	Final Volume per well
7.5 µL	2.5 µL	10 µL
9.0 µL	3.0 µL	12 µL
10.5 µL	3.5 µL	14 µL
12.0 µL	4.0 µL	16 µL
13.5 µL	4.5 µL	18 µL
15.0 µL	5.0 µL	20 µL

SDS-PAGE and Western Blot

1. Heat sample at 95°C for 3 minutes (or 70°C for 10 minutes)
2. Load equal volumes in each gel lane.
3. Separate proteins by SDS-PAGE.
4. Transfer proteins to immobilizing membranes.
5. Perform Western blot as per standard protocols using IRDye® conjugated secondary antibodies to detect target proteins.
6. Image membrane on Odyssey Classic, CLx or Fc in 700 and 800 channels.

Important: After imaging, visual assessment of band intensity can be misleading. Bands should be quantified utilizing Image Studio™ Software to determine differences between samples.

Software Analysis with Image Studio™ Software 5.x

Depending on the application of the Odyssey Loading Indicator data analysis may vary. A video tutorial for data analysis using Image Studio™ Software 5.x is available at licor.com.

Protocols for specific applications can be found at licor.com/support.