# *Manual for 5x Lysis Buffer*

Trade name: **5x Universal Lysis Buffer**
Product #: 333

## Product describtion

| | |
|---|---|
| Product name: | 5x Universal Lysis Buffer |
| Product No.: | 333 |
| Manufacturer: | Prolume / NanoLight |
| Quantity: | 50 ml |
| Buffer systems: | Use with the following buffer systems:<br>Cat. # 318 Firefly Assay Reagent<br>Cat. # 324 NLuc FLASH buffer |
| Storage conditions: | Long term storage at -20°C. Short term (several months) at 4°C. Let product come to room temperature before use. |

## Manual

1. Although this is a 5x concentrated lysis buffer, we recommend adjusting the concentration according to your cell line system. See the table below:

| Cell Line | Recommended Lysisbuffer dilution | Notes |
|---|---|---|
| HEK293/HEK293T | 20x to 5x | 10x for cytosolic lysis, 5x for stronger solubilization |
| HeLa | 10x to 5x | 5x for full protein lysis |
| CHO | 10x to 5x | Usually using the buffer as 10x works very well |
| NIH 3T3 | 5x | Fibroblasts, use as 5x |
| Jurkat / K562 | 20x | Very susceptible to lysis use less lysis buffer |
| C2C12 (muscle) | 5x to 2x | Tougher membrane, combine with freeze-thaw |
| Primary cells | 20x to 10x | Start with lower amounts first |

2. For a standard lysis solution prepare 1x lysis buffer by adding 4 volumes of double distilled water to 1 volume of 5x lysis reagent. For a milder lysis mix 9 volumes of double distilled water and 1 volume of 5x lysis reagent. Add to washed cells as followed.

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**Preparing Mammalian Cell lysates:**

1. Remove growth medium from cultured cells. Usually, high amounts of FCS will cause background luminescence with Coelenterazine based assays.
2. Carefully rinse cells with PBS or TBS. Remove wash solution completely.
3. Add the prepared 1x lysis reagent to your culture vessel to cover its surface. (e.g. 20 µl/well in 96 well plate, 400 µl per 6 cm culture dish, 900 µl for 10 cm culture dish)
4. For culture dishes, scrape adherend cells from dish and transfer into microcentrifuge tube. Pellet debris by brief centrifugation and transfer supernatant into a new tube.
5. Follow the instructions of your luciferase assay kit. In general mix equal amounts of lysate (e.g. 50 µl) with the luciferase assay buffer (e.g. 50 µl FLuc buffer + added D-Luciferin from the #318 kit).