# Iron oxidoreductase assay

## 1. Reaction Buffer

| | Final conc. (mM) |
|---|---|
| MES | 25 |
| MOPS | 25 |
| NaCl | 140 |
| Glucose | 5 |
| KCl | 5.4 |
| $CaCl_2$ | 1.8 |
| $MgCl_2$ | 0.8 |
| Adjust pH to 7.4 | |

###MES and MOPS can be replaced with 16,7 mM HEPES.

**2. Fe(III)-NTA3 (***iron*(III)-nitrilotriacetate (*NTA*)**) stock solution (1 mM Fe(III) plus 3 mM NTA)**

***Ferric nitrate (FeNO3) or FeCl3 was dissolved in 100 mM HCl. Disodium nitrilotriacetate (Na2NTA) was dissolved in H2O (If Nitrilotriacetic acid is used, 10 M NaOH solution is needed to help NTA acid dissolution). The respective solutions were mixed to achieve a molar ratio of 1:3 of Fe-NTA. The pH was adjusted to 7.4 with sodium bicarbonate with constant stirring (I prepared Fe-NTA stock solution at 10 mM). (Ref. *Awai M, Narasaki M, Yamanoi Y, Seno S. Induction of diabetes in animals by parenteral administration of ferric nitrilotriacetate. A model of experimental hemochromatosis. Am J Pathol 1979;95:663–72.*)

## 3. Ferrozine

Desired amount of ferrozine can be dissolved in either H2O or Reaction Buffer (without **Fe-NTA3**) just before use. Keep the solution in dark.

**Procedures:**

1. Transfect cells (in 6-well or 12-well plate) with target DNA

2. Forty-eight hours after transfection, cells are washed once with phosphate-buffered saline (PBS), pH 7.2.

3. Prepare Iron Uptake Buffer (IUB, containing 50-100 µM Fe-(NTA)3 and 1 mM (at least 200 µM) ferrozin) by combine Reaction buffer, ferrozine and **Fe-NTA3.**

**4.** Incubate cells IN DARK in Iron Uptake Buffer (800 µl/well of 12-well plate) 37 °C for 20-60 min. (*** Once ferrozine is mixed with Fe-NTA and buffer, the mixture must be kept from light.)

5. After incubation, the $Fe^{2+}$-ferrozine complex was detected by measuring the absorbance at 562 nm and converted to $Fe^{2+}$ generated by using the extinction coefficient 27.9 $mM^{-1}cm^{-1}$.

Cell number : Normally an 80% confluent well of HEK293T cells in a 6 or 12 well plate should give you a reliable assay after 1 hr. incubation.

**** Original protocol suggests that all reagents should be prepared before use. In my experience, **Fe(III)-NTA3 can be stored in fridge for several months. Reaction buffer (without Fe(III)-NTA3 and ferrozine can be kept at -20 degree for very long time. Normally 4x reaction buffer is prepared to minimize solution volume for storage. )**