# **Gus Assay Protocol**

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## 1. Stock Solutions

#### 50mM K<sub>3</sub>Fe(CN)<sub>6</sub> (50ml)

- Dissolve 0.823g in ~40ml H<sub>2</sub>O
- Bring to final volume

Note: Potassium ferricyanide (MW=329.25) should be stored in brown bottles at room temperature and should be made freshly each month. **Disposal**-Add an equal volume of Clorox and let stand overnight, then they may be washed down the drain with lots of water. Cyanides are toxic!

## K<sub>4</sub>Fe(CN)<sub>6</sub> 3H<sub>2</sub>O (50ml)

- Dissolve 1.056g in  $\sim 40$  ml H<sub>2</sub>O
- Bring to final volume

Note: Potassium ferrocyanide (MW=422.1) see above note.

#### 0.5M Na·EDTA·2H<sub>2</sub>O (100ml, MW=372.2)

- Dissolve 18.61g in  $\sim 80$  ml H<sub>2</sub>O
- Adjust pH to 8.0 ~ \_\_\_\_g of NaOH. *Note: This is necessary to dissolve the EDTA*).
- Bring to final volume.

#### 0.2M Monobasic NaH<sub>2</sub>PO<sub>4</sub> (1L, MW=120)

• Dissolve 24g in H<sub>2</sub>O and bring to final volume

#### 0.2M Dibasic Na<sub>2</sub>HPO<sub>4</sub> (1L, MW=142)

• Dissolve 28.4g in H<sub>2</sub>O and bring to final volume

#### Sodium Phosphate Buffer (pH 7.0 by definition)

• Mix 32 ml of monobasic stock and 68 ml of the dibasic stock.

#### 10% Triton X-100

- Dilute 10 ml of Triton X with 90 ml H<sub>2</sub>O
- Stir as necessary to dissolve into solution.

## 2. Reaction Mix (10ml)

•	Potassium ferricyanide (50nM)	1ml
•	Potassium ferrocyanide (50nM)	1ml
•	Sodium Phosphate Buffer	5ml
•	Sodium EDTA (0.5 M)	20µ1
•	Triton X (10%)	1ml
•	H <sub>2</sub> O	780 µl

# 3. X-Gluc

Dissolve X-Gluc (5-Bromo-4-Chloro-3-Indoyl-Beta-D-Glucuronide) in N,N-Dimethylformamide at a concentration of 25mg/ml.

Note: N,N-Dimethylformamide will dissolve plastic. It is toxic and should be handled in an exhaust hood. Pipet tips should also be disposed of in a temporary disposal container stored in an exhaust hood.

## 4. Reaction mix

Mix the reaction mix with X-Gluc at a ratio of 352µl of reaction mix to 48 µl of X-Gluc.

Approximate volumes:	96 well dish-100 $\mu l,$ 24 well dish-500 $\mu l,$
	35mmdish 0.7-1.0ml, 600mm dish 1.4ml

#### 5. Incubation

Incubation times should be determined empirically as results will vary depending on the tissue type, the promoter being tested (weak vs strong) and penetrability of the reactants to the site of expression. The incubation should be best understood as similar to developing film. Overexposure (too long an incubation time) will result in artifacts (especially with a strong promoter, such as CaMV 35S or Ubiquitin) as well as underexposure (too short an incubation time) may result in lack of detection (especially with a weak or tissue specific promoter) The recommended time in most publications to incubate the tissue in the Gus assay mix is for 4-16 hours at 37°C or longer at 25°C.