



GFP “Fold ‘n’ Glow” Assay Kit

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RESEARCH PRODUCT INSERT

INTENDED USE

The Fold n’ Glow kit allows a test protein to be expressed as an N-terminal fusion with GFP and will determine the amount of protein that is properly folded in a given sample as the folding reporter gives a signal directly proportional to the amount of correctly folded protein¹. The kit can be used for the detection and quantification of any protein by tagging and detecting either soluble or insoluble proteins. The kit contains sufficient reagents for one 96-well plate (96 tests).

BACKGROUND AND PRINCIPLE OF THE TEST

Background:

Green fluorescent protein (GFP) fusions and split protein tags are widely used for the analysis of protein. These large tags can perturb protein solubility, misfold, and alter the protein’s processing. The split GFP technology used in the Fold ‘n’ Glow assay overcomes these problems. The protein tag is a genetically encoded split GFP technology, engineered with small, soluble, self-associating GFP fragments. Thus, it is a simple split GFP system that doesn’t change fusion protein solubility, or require chemical ligation, fused interacting partners, co-expression or co-refolding. Furthermore, while fluorogenic biarsenical FlaSH or ReASH substrates also overcome these limitations, they also require many other conditions not necessary when using the split GFP technology. The GFP system is a simple and easy tagging and detection system². This kit may be used: to quantify the expression level of the tagged protein; to determine the solubility of a protein; or to determine the solubility of a protein’s domain.

The assay:

The kit is a protein tagging and detection method that uses split GFP technology in a fluorescent complementation assay. The protein to be quantified is fused to a small GFP fragment (contained in the S11 plasmid) via a flexible linker. Expressed separately, neither the fusion protein of interest or the GFP detector (S1-10) is fluorescent. When mixed, the properly folded fusion protein and detector spontaneously associate, formatting the fluorophore. Misfolding or aggregation of the fusion protein make the GFP tag inaccessible and prevent complementation, thus preventing fluorescence. Therefore, misfolded or aggregated proteins are not included in the quantification of the protein of interest.

REAGENTS

Components Supplied: *(Sufficient reagents have been supplied for 84 tests)*

Detector (S1-10): Complementary GFP fragment. Supplied ready to use. Store 2-8°C.

Positive control (SRS11): Positive control GFP fusion protein. Supplied ready to use at 5mM. Store 2-8°C.

Plasmid (S11) : Kanamycin resistance (Kan^R). *Bam*HI and *Nde*I restriction sites. Supplied at 100ng/ml Store -20°C.

Note: The shelf life of this kit is estimated to be at one month.

Reagent Storage: Store all reagents between 2° and -20°C as listed on each kit component. When stored properly, the reagents are stable until the date indicated either on the box or each component. Depending on the particular usage requirements, it may be appropriate to re-aliquot reagents to smaller working volumes to avoid repeated freeze-thawing or repeated pipetting from the same vial.

Materials required, but not supplied:

• Competent expression cells (BL2/DE3)	• 4°C refrigerator
• Kanamycin	• -20°C freezer
• IPTG (Isopropyl β -D-1-thiogalactopyranoside)	• Incubator
• LB growth media and plates	• Centrifuge(s) and appropriate size tubes
• <i>NdeI</i> And <i>BamHI</i> restriction enzymes	• Sonicator
• Ligation materials	• Microplate fluorescence reader
• Metal affinity column	• Vortex mixer
• TNG Buffer (50mM Tris pH 7.4, 0.1M NaCl, 10% glycerol)	• Water bath
• Bovine serum albumin	• Graduated cylinders and assorted beakers
• Plasmid Isolation Reagents	• Pipettes and tips
• 96 well plate	• Disposable gloves

A. Preparation of insert DNA

Prior to performing the assay, carefully read all instructions.

1. Perform plasmid prep and/or PCR. Use standard materials/protocol not included.
2. Use *NdeI* (5') and *BamHI* (3') restriction sites to perform a restriction digest that generates overhangs on the DNA insert.
3. *Optional*: Purify digest fragment from agarose gel.

B. Preparation of S11 Vector

1. Perform restriction enzyme digest of the S11 vector using *NdeI* and *BamHI* to prepare the plasmid for the insert DNA. Follow the manufacturer's instructions for use of the enzymes. Leave sticky ends overhangs in preparation for ligation.
2. *Optional*: Dephosphorylate the digest to decrease non-recombinant background. Use molecular grade calf intestinal or shrimp alkaline phosphatase according to the manufacturer's directions.
3. Perform ligation reaction according to manufacturers' instructions.
4. Store vector at -20°C until used.

C. Clone DNA insert as an N-terminal fusion into S11 vector

1. Ligate the DNA insert with the digested S11 using standard DNA ligation protocol and manufacturer's protocol resulting in a S11 fusion plasmid.
2. Transform the S11-fusion plasmid in an expression host for high yields of quality plasmid. Use standard methods based on the screening host used.
3. Identify the positive clones using standard methods. Note: IPTG / X-gal screening is effective in the first 24 hours post plating as the T7 promoter is highly active and absorbs resources from the LacZ gene.
4. Perform plasmid DNA purification, sequence to verify reading frame, or use *in vitro* transcription/translation.

D. Preparation of S11 fusion protein

Prepare cells and extract *soluble* S11 fusion proteins.

Grow a 200ml culture of bacteria, transformed S11 fusion protein, in LB growth medium and Kanamycin [20-50mg/ml] to log phase at OD_{600nm} of 0.5-0.8.

Induce with 1mM IPTG for 4hr at 37°C.

Pellet cells by centrifugation and re-suspend in 2ml TNG buffer.

Sonicate to disrupt bacterial cell walls to release soluble proteins.

Optional:

Purify *soluble* S11 fusion protein by metal-affinity column.

Determine the purity and quantify protein.

Store the fusion proteins at -20°C until *In vitro* complementation assay is performed.

E. In Vitro Complementation assay

Prepare 96-well plate(s)

Block 96-well flat bottom microplate with 0.5% w/v bovine serum albumin in TNG, 10 min.

Serially dilute positive control reagent 22µl:22µl in TNG buffer from 5µM-39nM, or lower depending on instrument sensitivity.

Add 20µl of each standard dilution to the first two columns of a 96-well plate (see diagram).

Add 20µl aliquots of the protein fusion(s) prepared in part C. to remaining wells in the same 96-well plate.

Prepare a negative control of 0.5% BSA in TNG buffer and add 20ul to the plate.

Perform complementation by adding 180µl aliquot of GFP 1-10 to all of the above wells except the blank.

RESEARCH DATA ANALYSIS

Data Analysis

Subtract the blank fluorescence values from the final fluorescence values of the sample(s) and the positive control. Estimate protein concentration by comparing fluorescence on the standard curve.

REFERENCES

1. Waldo et al. "Rapid protein-folding assay using green fluorescent protein," *Nature Biotechnology* 17, 691 - 695, July 1999.
2. Cabantous et al. "Protein tagging and detection with engineered self-assembling fragments of green fluorescent protein," *Nature Biotechnology* 23, 102-107, December 2004.

Suggested Plate Configuration

	1	2	3	4	5	6	7	8	9	10	11	12	
A	Neat positive control	Neat positive control	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	
B			Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	
C			Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	
D			Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	
E			Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	
F			Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	
G	▼	▼	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Blank	Blank
H	.39nM positive control	.39nM positive control	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	BSA negative control	BSA negative control

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