F2H®-Kit Basic for Analyses of Protein-Protein Interactions in Live Cells

For fast and convenient characterization of interactions between GFP- and RFP-tagged proteins in live mammalian cells by conventional fluorescence microscopy.

Only for research applications, not for diagnostic or therapeutic use.

Introduction

Intracellular analysis of protein-protein interactions (PPIs) is crucial for understanding of intimate relationships of proteins within their native cellular environment. Besides, cell-based analysis is often required to support biochemical data obtained with *in vitro* PPI assays.

With the Fluorescent Two-Hybrid (F2H®) Kit, evaluation of selected PPIs can be rapidly carried out in live mammalian cells. Interactions are visualized by simple fluorescence microscopy as co-localization of green- and red fluorescent signals at a PPI-platform in the nucleus of F2H®-Cells, transiently co-transfected with interacting GFP- and RFP-tagged proteins.

Content

Reagent	Quantity
f2h-bhk, F2H®-Cells genetically engineered BHK cells stably expressing components of the PPI-platform	1 vial, 5 X 10^6 frozen cells in serum-free cryopreservation media (DMSO 8.7%)
f2h-p100, Platform Reagent (red cap) transfection supplement enabling assembly of the nuclear PPI-platform	1 vial, 100 µL, 1 mg/mL, for 125 transfections in 24-well format or 500 transfections in 96-well format
f2h-c30, Control-DNA (yellow cap) plasmid mixture of interacting GFP-bait and RFP-prey (positive control)	1 vial, 30 µL, 1 mg/mL, for 37 transfections in 24-well format or 150 transfections in 96-well format

Stability and Storage

Shipped on dry ice. Upon receipt store frozen F2H®-Cells in -80°C (short term, days) or in liquid nitrogen (long term, months). Store Platform Reagent and Control-DNA at +4°C (short term) or in -20°C (long term).

All kit components are stable up to six (6) months from the date of receipt if stored and handled correctly.

Further Reagents Required

- Plasmids coding for your GFP- and RFP-tagged proteins of interest are required for performing F2H®-Assays (to be generated by the investigator, see guidelines below).
- Transfection reagent, complete growth medium, trypsin, DPBS are not provided.

Guidelines for the Plasmids

To analyze interactions between the two proteins of interest further named ProteinX and ProteinY, cDNAs of these proteins or their domains should be cloned into mammalian expression vectors (such as pcDNA3.1, pEGFP-N1, pTagRFP-C):

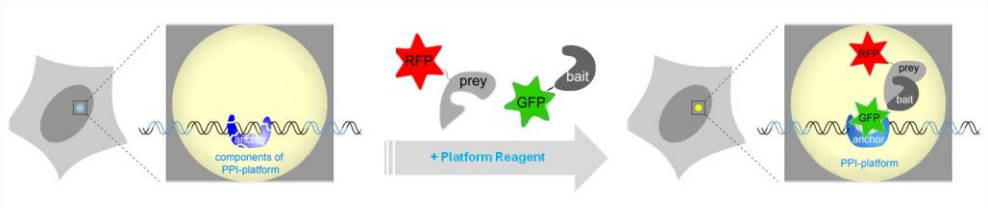
- One protein should be in-frame with GFP (or eGFP, YFP, CFP, Venus, TagGFP2). This **GFP-ProteinX** will serve as **bait**.
- The other protein should be in-frame with **RFP** (or mRFP, mCherry, mOrange, TagRFP, mKate). This **RFP-ProteinY** will serve as **prey**. Monomeric red fluorescent proteins are preferred, we recommend extremely bright TagRFP (Evrogen, Russia).

Optional: To enrich the fusion proteins in the nucleus, the fusion constructs may be preceded with a nuclear localization signal (NLS). In most cases NLS is not necessary for the assay.

Note 1: It is advised to verify the expression levels and subcellular localization of the fusion proteins in mammalian cells prior to performing F2H®-Assays. High transfection efficiency, good expression, appropriate subcellular localization and the absence of aggregation are the criteria to take care of when working with fusion proteins.

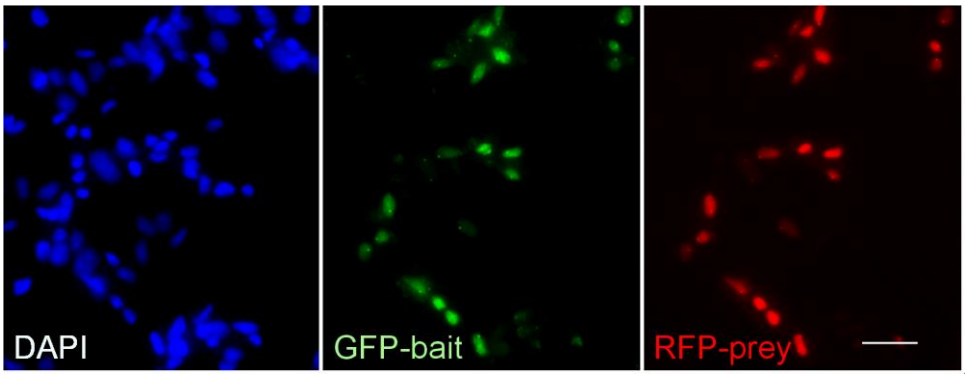
Note 2: It might be helpful to test fusions to different fluorescent proteins, compare N- vs. C-terminal fusions or fuse just interacting domains of the proteins with the fluorescent proteins.

Assay Principle

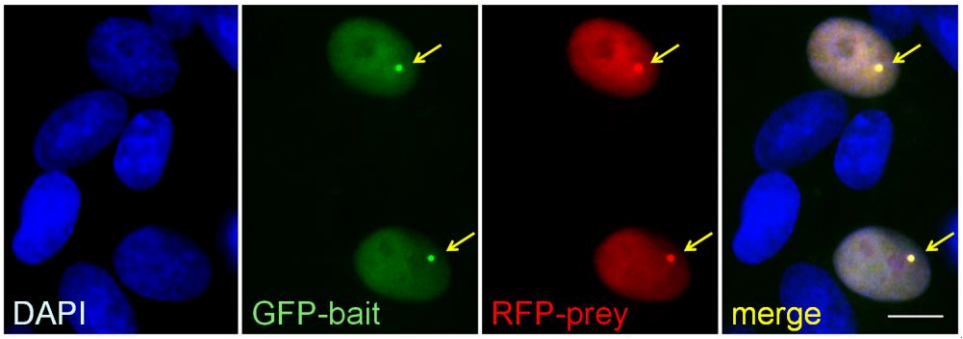


- F2H®-Cells stably express components of the PPI-platform;
- GFP-bait and RFP-prey are co-transfected into F2H®-Cells;
- Interactions are imaged one day after transfection:
  - co-localizing **green** and **red** "spot" in the nucleus → interaction,
  - only **green** "spot" (red is diffuse) → no interaction.

Assay Performance



An overview image of co-transfected F2H®-Cells at 20X magnification. Cells were PFA-fixed, nuclei were stained with DAPI. Co-transfection efficiency is ~20% in this experiment. Scale bar, 40 µm.



F2H®-Cells were co-transfected with interacting proteins, fixed, stained with a nuclear dye (DAPI) and imaged using a 63X objective. Yellow arrows point at the interactions, which are clearly visible at this magnification. Scale bar, 10 µm.

Related Products

Use our GFP-Trap® and RFP-Trap® for biochemical analyses of protein-protein interactions by one-step immunoprecipitation of fusion proteins from cell lysates.

Support

Please contact *support@chromotek.com* or call +49 89 78797310.

Limited Use Label License

F2H®-Kit Basic: Protocol

Only for research applications, not for diagnostic or therapeutic use.

Part I: Culture F2H®-Cells

> Use aseptic technique for sterile handling of cell cultures when working with F2H®-Cells.

> F2H®-Cells are genetically modified Baby Hamster Kidney fibroblasts (BHK) and can be cultured according to standard protocols for maintenance of BHK cells.

Thaw F2H®-Cells

> Prepare complete growth medium (not provided) prior to thawing procedure:

Complete growth medium
• Dulbecco's Modified Eagle's Medium (DMEM) with 4.5 g/L glucose, 110 mg/L sodium pyruvate and L-gluthamine • 10% Fetal bovine serum (FCS) • 50 µg/mL Gentamycin

> Remove frozen cells from storage and thaw quickly in +37°C water bath.

> **Immediately** upon thawing transfer thawed cells (~1.5 mL) into a Falcon tube containing 10 mL of complete growth medium. Mix gently and centrifuge at ~80 *g* for 3 min.

> Aspirate supernatant without disturbing the pellet.

> Gently resuspend pelleted cells in 1 mL of complete growth medium and transfer into a 100-mm cell culture dish, containing 10 mL of complete growth medium.

Note: To culture F2H®-Cells, we use Falcon® 100-mm polystyrene cell culture dishes with standard tissue culture/gas plasma treated surface (Ref. 353003, Corning, USA).

Subcultivate F2H®-Cells

> Culture cells in a humidified +37°C, 5% CO_2 incubator. Check daily if cells are confluent. When confluent, subculture (split) by trypsinization as outlined below.

> Briefly wash cells with Dulbecco's Phosphate Buffered Saline (DPBS, 1X) without Ca & Mg supplemented with 0.5 mM EDTA.

> Aspirate and add 0.5 mL Trypsin/EDTA onto cells for ~3 min at 37°C.

Note: We use 0.05% Trypsin / 0.02% EDTA in DPBS or HBSS, e.g. 1XTrypsin-EDTA Solution (T3924, Sigma, USA).

> When cells are loose, add 10 mL complete growth medium to the plate, resuspend gently. Use light microscopy to check that the cells are well resuspended. Plate trypsinized cells 1:3 – 1:30 (see table below):

Subcultivation ratio for F2H®-Cells (starting with confluent cultures)
• Plate cells 1:25-1:30 if to be kept longer in culture (at this splitting ratio cells should be subcultivated at least twice a week, maximum 25 passages recommended). • Plate cells 1:5 if to be transfected upon splitting (e.g. by reverse transfection, recommended for 96-well plates). • Plate cells 1:10 if to be transfected the next day (recommended for coverslips).

Tipp 1: Do not let the cells overgrow! Do not plate them too thin either (max. splitting ratio 1:30).

*Tipp 2: **Cell density is critical** for transfection efficiency! If transfecting BHKs for the first time, try several densities, e.g. 1:3, 1:6, 1:12. For reverse transfection, pre-splitting cells the day before (1:3 – 1:5) increases transfection efficiency.*

Part II: F2H®-Assay

On Day 1 F2H®-Cells are co-transfected with the bait and the prey. On Day 2 interactions are imaged.

Day 1:

Transfect F2H®-Cells

> Co-transfect your GFP- and RFP-tagged proteins (plasmid ratio 1:3, w:w) into F2H®-Cells. Use a transfection reagent appropriate for BHK cells according to manufacturer's instructions. We recommend Lipofectamine® 2000 (Life Tech., USA).

> During the transfection: To ensure assembly of the PPI-platform in the nucleus, always pre-mix your plasmids with the same amount of the **Platform Reagent (red cap)** before adding a transfection reagent. Thus, if 1 µg total DNA is required for transfection, pre-mix it with 1 µg Platform Reagent.

Example: Transfection in a 24-well plate format.

Seed F2H®-Cells on coverslips a one day in advance. Cells should be ~25% confluent at the time of transfection. Per well, 0.8 µg total DNA and 1.6 µL Lipofectamine® 2000 are required:

- *Pre-mix the components in two tubes as specified in the tables on the right.*
- *Combine the content of the tubes, mix gently.*
- *Let stand for 5-20 min at room temperature.*
- *Add dropwise into the well containing cells and media.*
- *Place the plate in the incubator.*

Pre-mix in one 1.5 mL tube:	
50 µL	*DMEM (without FCS & Gentamycin)*
0.2 µg	*Plasmid DNA GFP-ProteinX*
0.6 µg	*Plasmid DNA RFP-ProteinY*
0.8 µg	***Platform Reagent (red cap)**, 1 µg/µL*

Pre-mix in another 1.5 mL tube:	
50 µL	*DMEM (without FCS & Gentamycin)*
1.6 µg	*Lipofectamine® 2000*

Note: To scale the transfections down to 96-well or up to 12-well formats, check the manufacturer's instructions for the transfection reagent you use.

> **Controls:** Each experiment should include at least 3 transfections: positive control, negative control and experimental sample.

> **Positive control:** Transfect the cells with the **Control-DNA (yellow cap)** along with the **Platform Reagent (red cap)**.

Example: Positive control transfection mixes for 1 well in a 24-well plate:
Tube 1: 50 µL DMEM + 0.8 µg Control-DNA + 0.8 µg Platform Reagent;
Tube 2: 50 µL DMEM + 1.6 µg Lipofectamine® 2000.

> **Negative control:** Co-transfect the cells with the plasmid RFP-ProteinY and a GFP-expression vector (GFP not fused to ProteinX). Do not forget to add the Platform Reagent! This verifies that RFP-ProteinY is not recruited to the Platform or GFP unspecifically.

Day 2:

Detect Interactions

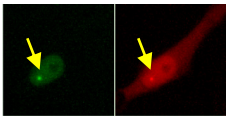
> Analyze interactions 16-24 h after transfection in living or fixed cells. For better image quality, we recommend fixing cells with 4% formaldehyde in PBS (10 min at RT) and staining nuclei with **DAPI**.

> Use a 40X objective and appropriate filter sets to detect your fluorescent proteins.

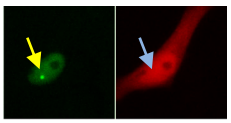
> Examine nuclei of co-transfected cells in green channel to find GFP-tagged bait anchored at PPI-platforms → identify one, rarely two bright green "spots" per nucleus.

> Switch to the red channel and check for accumulations of red fluorescence at the locations corresponding to the green "spots":
• **Red** "spot" co-localizes with a **green** "spot" → **interaction**,
• No red "spot" (the cell is co-tranfected and has a **green** "spot") → **no interaction**.

Interaction:



No Interaction:



> ***Positive control:*** *Co-localizing green and red "spots" should be detectable in ~90% of cells co-transfected with the Control-DNA mixture in presence of the Platform Reagent.*

> ***Negative control:*** *Cells that are co-transfected with GFP and RFP-ProteinY (along with the Platform Reagent) should show green "spots", but no or very few co-localizing red "spots".*