# Human IL10 TR-FRET Detection Kit

Instructions for Use | Cat. No.: ARK0230

## 1. Principle

This kit quantitatively detects Human IL10 in samples such as cell culture supernatant. It is a sandwich immunoassay based on TR-FRET (time-resolved fluorescence resonance energy transfer). A pair of specific antibodies is labeled with the fluorescent donor Solar Eu and the fluorescent acceptor LA, respectively. When both antibodies bind to different epitopes of Human IL10, excitation of the donor triggers energy transfer to the acceptor; the signal at 665 nm is proportional to the amount of Human IL10 in the sample.




Figure 1. Principle of the TR-FRET cytokine detection. Donor: Solar Eu; acceptor: LA; Ag: cytokine.

## 2. Components

| Component | Storage | 100 tests | 1,000 tests | 10,000 tests |
|---|---|---|---|---|
| mAb anti-IL10-Solar Eu (400X) | ⩽ -60 °C | 1×5 µL | 1×50 µL | 1×500 µL |
| mAb anti-IL10- LA (400X) | ⩽ -60 °C | 1×5 µL | 1×50 µL | 1×500 µL |
| Human IL10 Standard (30 ng/mL) | ⩽ -60 °C | 1×50 µL | 2 vials (lyo) | 2 vials (lyo) |
| Cytokine Diluent Buffer (ready-to-use) | 2–8 °C | 2×2 mL | 1×20 mL | 1×50 mL |
| Cytokine Detection Buffer (ready-to-use) | 2–8 °C | 1×2 mL | 1×10 mL | 1×50 mL |

* Test number (tests) refers to the number of wells that can be run in a 96- or 384-well plate with a 20 µL total reaction volume at the recommended reagent concentrations. 400X is the stock concentration; the final concentration in the 20 µL reaction is 1X.

## 3. Storage

- Store all components under the conditions indicated on their labels. Product quality is guaranteed for 1 year from the date of receipt.
- Human IL10 Standard must be stored at ⩽ -60 °C; the detection antibodies must be stored at ⩽ -60 °C. Aliquot on first use and avoid repeated freeze-thaw cycles; aliquot volumes of at least 10 µL are recommended.

## 4. Materials Required but Not Provided

| Material | Recommended source |
|---|---|
| Low-volume white 96-well microplate | AREX Biosciences |
| Low-volume white 384-well microplate | AREX Biosciences |
| Transparent pressure-sensitive plate seal (readable without removal) | AREX Biosciences |
| Microplate reader with TR-FRET capability | Various |

## 5. Assay Procedure

### 5.1 Reaction System

| Step | Volume (total 20 µL) | Preparation |
|---|---|---|
| 1. Sample | 16 µL | Test sample or working standard |
| 2. Detection reagent | 4 µL | 5X premixed detection antibody pair |

* The 20 µL system is intended for low-volume 384-well plates. Volumes may be scaled proportionally for 96-well or 1536-well plates.

### 5.2 Reagent Preparation

- Equilibrate all reagents to room temperature before use and mix liquid reagents gently.
- Reagents stored at ⩽ -60 °C should be thawed on ice and equilibrated to room temperature before use.
- Dilute and prepare all reagents with the buffers supplied in the kit.
- Prepare all working solutions immediately before use.
- Gently rock all reagents to mix. Do not vortex.

### 5.3 Working Solution Preparation

**Standard curve.** Prepare the standard dilution series with Cytokine Diluent Buffer or with a solution of the same matrix as the test samples. Using the sample matrix for the standard curve is recommended to reduce matrix effects. The volumes below are for reference only.

| Standard | Working conc. (pg/mL) | Final conc. in 20 µL (pg/mL) | Preparation |
|---|---|---|---|
| STD-7 | 6,000.0 | 4,800.0 | 40 µL standard stock + 160 µL Diluent Buffer |
| STD-6 | 2,608.7 | 2,087.0 | 100 µL STD-7 + 130 µL Diluent Buffer |
| STD-5 | 1,134.2 | 907.4 | 100 µL STD-6 + 130 µL Diluent Buffer |
| STD-4 | 493.1 | 394.5 | 100 µL STD-5 + 130 µL Diluent Buffer |
| STD-3 | 214.4 | 171.5 | 100 µL STD-4 + 130 µL Diluent Buffer |
| STD-2 | 93.2 | 74.6 | 100 µL STD-3 + 130 µL Diluent Buffer |
| STD-1 | 40.5 | 32.4 | 100 µL STD-2 + 130 µL Diluent Buffer |
| STD-0 | 0.0 | 0.0 | 130 µL Diluent Buffer |

**Sample preparation.** Dilute samples with Cytokine Diluent Buffer or the same matrix solution into the quantifiable range (105 – 6,000 pg/mL). Use the same diluent for samples and standards.

**Detection antibodies.** Dilute mAb anti-IL10- Solar Eu (400X) and mAb anti-IL10- LA (400X) each 40-fold with Cytokine Detection Buffer to obtain the working solution (2 µL per well). Mix equal volumes of the two working solutions to prepare the 5X detection antibody pair premix (4 µL per well).

### 5.4 Assay Procedure

| Step | Negative control | Standard curve | Sample |
|---|---|---|---|
| 1 | 16 µL Diluent Buffer | 16 µL diluted standard | 16 µL prepared sample |
| 2 | Add 4 µL of the 5X detection antibody pair premix to each well | | |
| 3 | Seal the plate with a transparent pressure-sensitive film and incubate at room temperature for 1–3 hours at room temperature | | |
| 4 | Read the plate on a TR-FRET compatible microplate reader without removing the film | | |

### 5.5 Data Processing

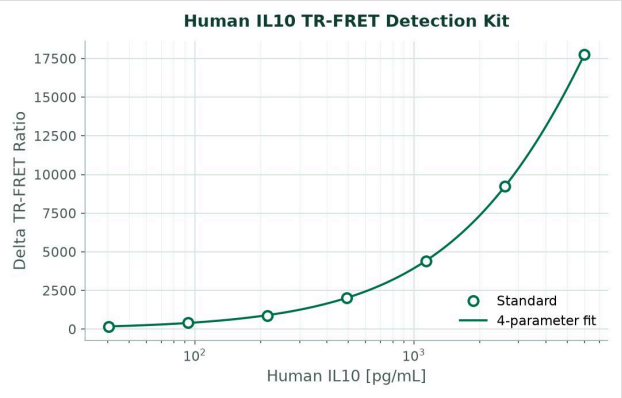
Calculate the TR-FRET ratio and the percentage CV (CV%) for each well:

TR-FRET Ratio = Signal 665 nm / Signal 620 nm × 10,000

## 6. Results

### 6.1 Standard Curve

| Standard | Concentration (pg/mL) | TR-FRET Ratio | Delta Ratio | CV% |
|---|---|---|---|---|
| STD-7 | 6,000.0 | 18,267 | 17,747 | 2.1 |
| STD-6 | 2,608.7 | 9,765 | 9,245 | 2.8 |
| STD-5 | 1,134.2 | 4,927 | 4,407 | 3.4 |
| STD-4 | 493.1 | 2,553 | 2,033 | 3.4 |
| STD-3 | 214.4 | 1,404 | 884 | 5.3 |
| STD-2 | 93.2 | 943 | 423 | 3.3 |
| STD-1 | 40.5 | 683 | 163 | 4.9 |
| STD-0 | 0.0 | 520 | 0 | 1.0 |




### 6.2 Performance

| Parameter | Diluent buffer | RPMI1640 + 10% FBS | DMEM + 10% FBS | 100% FBS |
|---|---|---|---|---|
| Assay range (pg/mL) | 105 – 6,000 pg/mL | | | |
| LoD (Std 0 mean + 2×SD) | 24.46 pg/mL | 58.11 pg/mL | 58.10 pg/mL | 62.57 pg/mL |
| LoQ (Std 0 mean + 10×SD) | 105.42 pg/mL | 334.02 pg/mL | 232.13 pg/mL | 404.58 pg/mL |
| Incubation time | 2 hours at room temperature | | | |

* Sensitivity data were obtained on the specified reader under optimized settings; results may vary with instrument performance.

### 6.3 Instrument Settings

| | |
|---|---|
| Vendor | TECAN |
| Instrument model | Infinite® 200 PRO |
| Mode | Fluorescence Top Reading |
| Excitation filter | 320 (25) nm |
| Emission filter 1 | 665 (8.5) nm |
| Emission filter 2 | 620 (10) nm |
| Mirror | Dichroic 510 |
| Lag time | 150 µs |
| Integration time | 500 µs |
| Number of reads | 5 or user-defined |
| Gain | 150 or optimal |
| Z-focus (mm) | Calculated on the well with the highest signal |