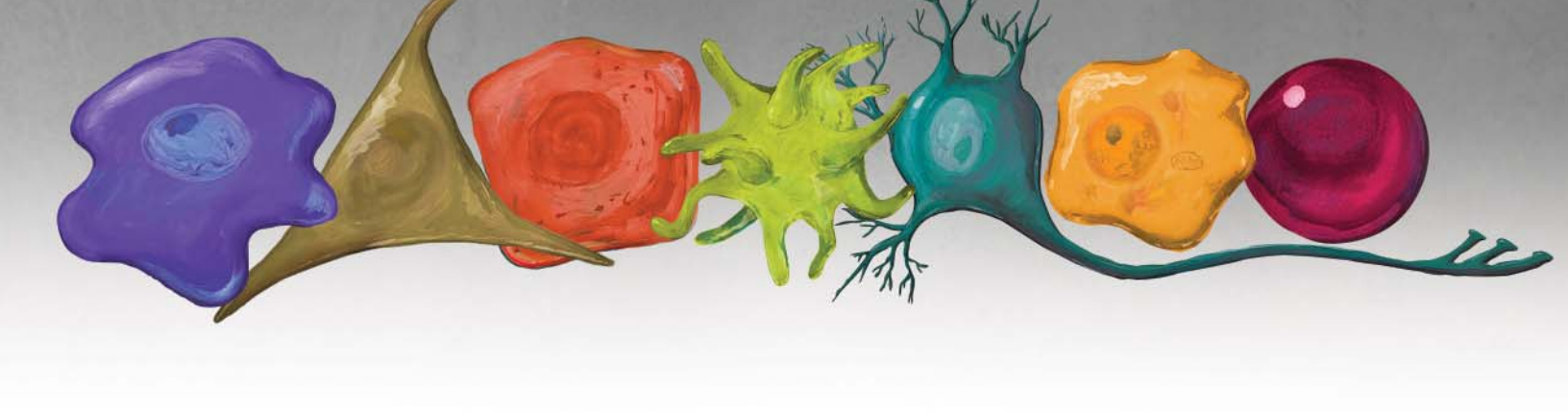

# R&D Systems

Committed to providing a comprehensive line of high quality **Tools for Cell Biology Research™**

## DuoSet® ELISA Development Systems

R&D Systems DuoSet ELISA development systems contain the essential components required to develop an immunoassay to measure natural or recombinant proteins. Each DuoSet kit contains the ELISA capture reagent, detection antibody, streptavidin-HRP, and a recombinant standard sufficient for running approximately fifteen 96-well plates.

### DuoSets are:

**Economical** – a cost-effective solution for running multiple ELISAs

**Optimized** – a detailed protocol reduces your preliminary development requirements

**Flexible** – can optimize coating concentration (& detection) to suit your particular needs

**Versatile** – streptavidin-HRP conjugate enables choice of chemiluminescent or colorimetric substrate

The product range includes assays for human, mouse, rat, canine, feline, porcine, equine, cotton rat, and primate:

- Cytokines
- Chemokines
- Growth Factors
- Proteases
- Adhesions Molecules
- Developmental Proteins

& more

**Please see www.RnDSystems.com/go/DuoSet**

### Colorimetric Assay Summary

| | | |
|---|---|---|
| **Step 1**<br>Coat microplate with Capture Antibody overnight in the supplied buffer and at the concentration specified by the insert | Wash plate and add block solution for 1 hour, minimum | Wash plate |
| **Step 2**<br>Add samples and standards to coated plate | Incubate for 2 hours | Wash plate |
| **Step 3**<br>Add biotinylated Detection Antibody | Incubate for 2 hours | Wash plate |
| **Step 4**<br>Add Streptavidin-HRP for 20 minutes and incubate<br>Wash plate | Add TMB substrate<br>Incubate for 20 minutes, then add stop solution | Read OD values at 450 nm/540 nm |


R&D SYSTEMS®

Quality | Selection | Performance | Results

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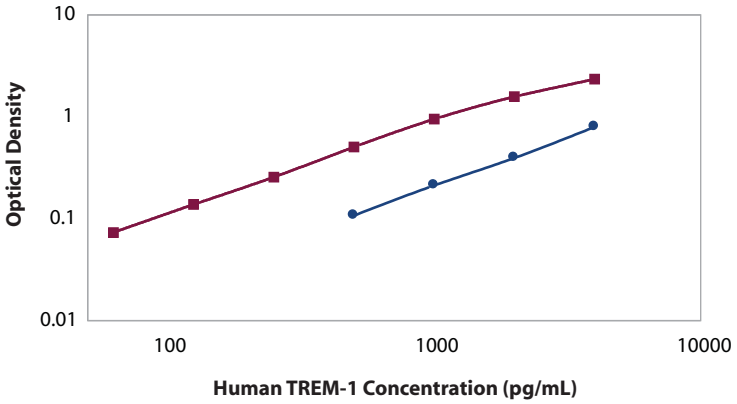
## Optimization

The detailed protocol is optimized for antibody concentrations, standard curve range, and buffers, thus saving lengthy optimization experiments.

Diluent formulations given in the inserts are suitable for most cell culture supernate samples. For assaying serum samples, each laboratory should develop and validate its own serum diluent. We suggest starting with PBS supplemented with 10–50% fetal calf serum. It is recommended that an ELISA be optimized for serum and/or plasma by spike recovery and dilution linearity experiments on a representative group of samples.




Human peripheral blood lymphocytes were treated with PHA (10 µg/mL) for 5 days. The supernatant was assayed neat and in a two-fold dilution series using R&D Systems human TREM-1 DuoSet ELISA (Catalog # DY1278) with colorimetric detection substrate (Catalog # DY999). The linear dilution of sample (blue) demonstrates that the DuoSet detects natural as well as the recombinant TREM-1 kit standard (red).

## Quality Control

Quality control testing on each new DuoSet lot ensures that the assay has:

- a blank reading of < 0.2 OD units
- a low delta of > 0.01 OD units
- a high signal of 1.5–3 OD units
- a correlation coefficient of > 0.997

## Specificity

Each DuoSet is tested for cross-reactivity with a panel of related molecules. These related molecules are also tested for their ability to interfere with correct analyte quantification. Details of cross-reactivity and interference tests are given in every DuoSet kit data sheet.

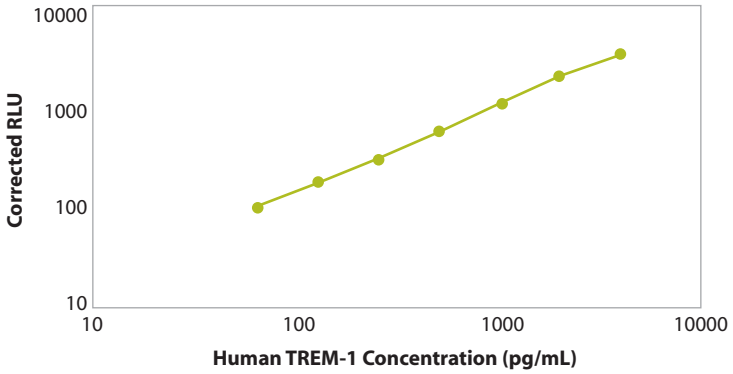
## Reproducibility

The protein standard provided in the kit is mass calibrated for immunoassays and eliminates lot-to-lot variation in concentration. Each new lot of DuoSet kits is tested using recombinant controls that must read within an established range.

## Versatility

DuoSets employ a streptavidin-HRP detection system which allows the freedom to choose one of several substrate options including chemiluminescence.

R&D Systems Glo Substrate Reagent Pack (Catalog # DY993) contains stabilized enhanced luminol and hydrogen peroxide, and can be used as a substitute for TMB in DuoSet ELISAs. Black microplates (Catalog # DY991) should be used for chemiluminescent assays.




R&D Systems human TREM-1 DuoSet (Catalog # DY1278) was developed using chemiluminescent detection substrate (Catalog # DY993) and black microplates (Catalog # DY991). Standard curve data are presented in relative light units (RLU).

## Supplemental Assay Development Products

| Supplemental Assay Development Products | Catalog # |
|---|---|
| Black Microplates, 25 Pack | DY991 |
| Clear Microplates, 25 Pack | DY990 |
| ELISA Plate Sealers, 100 Pack | DY992 |
| Glo Substrate Reagent Pack (Reagent A & B) | DY993 |
| Reagent Diluent Concentrate 1 | DY997 |
| Reagent Diluent Concentrate 2 | DY995 |
| Stop Solution 2N Sulfuric Acid | DY994 |
| Streptavidin-HRP | DY998 |
| Substrate Reagent Pack (Color A and Color B) | DY999 |

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