

The Ah-IMMUNOASSAY® (Ah-I®) kit* is designed to rapidly and economically screen for dioxin-like toxicity in environmental samples. The Ah-IMMUNOASSAY® presents new opportunities to monitor dioxin contamination and cleanup situations in a cost-effective manner. Dioxin-like compounds are defined as structurally related compounds with similar mechanisms of toxicity. The prototypical and most toxic dioxin is 2,3,7,8- tetrachlordibenzo-*p*-dioxin (commonly named 2,3,7,8- TCDD or just TCDD).

The Ah-IMMUNOASSAY® is to be used for screening for dioxin-like toxicity in environmental samples (e.g., soil, fly ash), and in biological samples (e.g. sera, tissue, food).

Dioxin-like compounds interact with a cellular protein, the Ah-receptor. The toxicity of these compounds, which can vary by one-thousand-fold depending on the congener, correlates to each congener's ability to interact with the Ah-receptor.



The standard method of assessing the dioxin-like toxicity of a test mixture is to separate the compounds by chromatography so that the identities and concentrations of each can be individually determined. The toxic contribution of each compound is calculated as a product of its concentration and its relative toxicity, called TEQ (Toxic Equivalents). The total toxicity of the matrix is the sum of these products. These methods are exquisitely sensitive, but expensive and labour-intensive. Thus, this standard assay method is not well adopted to high sample throughput screening.

The Ah-IMMUNOASSAY® fills this gap by providing the ability to screen a large number of samples quickly so that non-positives can be eliminated before subjecting the more interesting positive samples to further analysis. The Ah-IMMUNOASSAY® takes advantage of the correlation between the toxicity of dioxin-like compounds and their interaction with the Ah-receptor. The Ah-IMMUNOASSAY® measures the ability of the test mixture to interact with the Ah-receptor, thus providing data for the total toxicity of the mixture as 2,3,7,8-TCDD equivalents.



Sample extracts to be tested in the Ah-IMMUNOASSAY® are added to a reagent mixture containing Ah-receptor and other components in a special ELISA plate and allowed to incubate at room temperature for two hours. At that time, any Ah-receptor transformed by dioxin-like compounds is bound to the plate and the remaining material is washed away. Antibodies are added to the ELISA to detect the transformed, bound Ah-receptor which in turn is detected colorimetrically. Color development is proportional to the amount of transformed Ah-receptor.



*) The Ah-IMMUNOASSAY® is licensed from Paracelsian, Inc.



The detection limit of the Ah-IMMUNOASSAY® is one pg 2,3,7,8-TCDD equivalent per ELISA plate well. The linear range is 1-64 pg TCDD equivalents per ELISA plate well. About 2 µl of sample dissolved in DMSO is required per 200 µl ELISA plate well. The assay does not separate and identify single dioxin congeners but measures the ability of the test mixture to interact with the Ah-receptor, thus measuring the total toxic potential of the mixture as 2,3,7,8-TCDD equivalents (TEQ). Duplicate aliquots of each Reference Standard and sample dilutions are recommended. Replicate absorbance readings (OD) should show a coefficient of variation (CV) of ≤ 20%.

The broad sensitivity makes the Ah-I® an ideal toxicity screen. False negatives are rare. Positive results can be further characterized by selective pretreatment steps or by specific GC/MS testing.

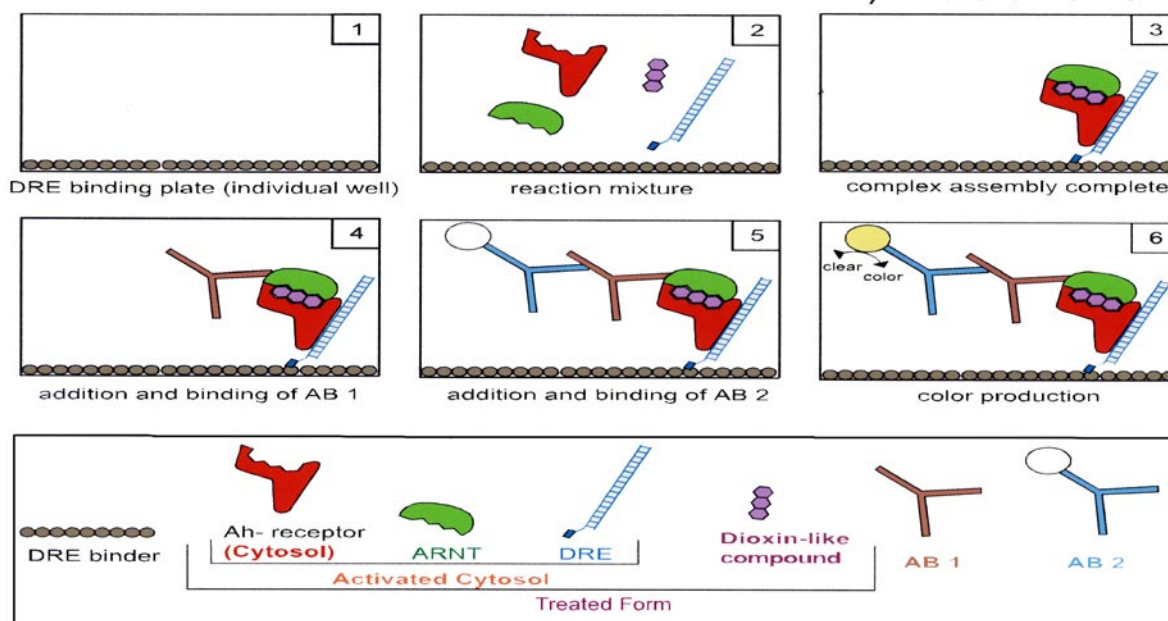
The Ah-IMMUNOASSAY® offers the best alternative to any screening method for dioxin-like toxins. It is:

- Broad in application (all dioxin-related toxins)
- Highly sensitive (parts per trillion levels in environmental samples)
- Easy to perform
- Inexpensive (allowing more extensive sampling)
- Reliable and reproducible
- Ideal as a dioxin screen

The activated Cytosol components (Cytosol, DRE Oligo, ARNT Extract, Activator) are shipped separately on dry ice. These components are stable for one month at -20° C and for three months at -80° C. The components (shipped at ambient temperature) are stable at +4° C for one year.

The Ah-IMMUNOASSAY® kit is available in a standard 96 well microplate format, that can be subdivided into 2-4 assays. Each kit can measure 20 samples in duplicate at two dilution points.

Pictorial View of Ah-Immunoassay Reactions



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