Chapter 2
Important Assay Details

Conducting an Elispot assay is a multistep process. The main steps are:

1. Choosing the correct reagents and materials
2. Preparing the sample
3. Running the assay
4. Acquiring the spot counts
5. Analyzing the data

Before we address the best choices for each step, let's have a look at what can be considered a perfect Elispot assay (Fig. 2.1).

A perfect Elispot has:

- Small, well-defined spots,
- Even spot distribution across the well,
- No artifacts,
- No background staining (elevated staining of the membrane),
- No or very low background reactivity (no or very few spots in negative control wells),
- No false-positive spots,
- Low variability between replicate wells,
- A working positive control,
- A trending control included,

and is

- Repeatable (precise),
- Accurate (measurement is close to the true value).

The next part of this book investigates in detail the necessary steps and choices that enable you to run a "perfect" Elispot.

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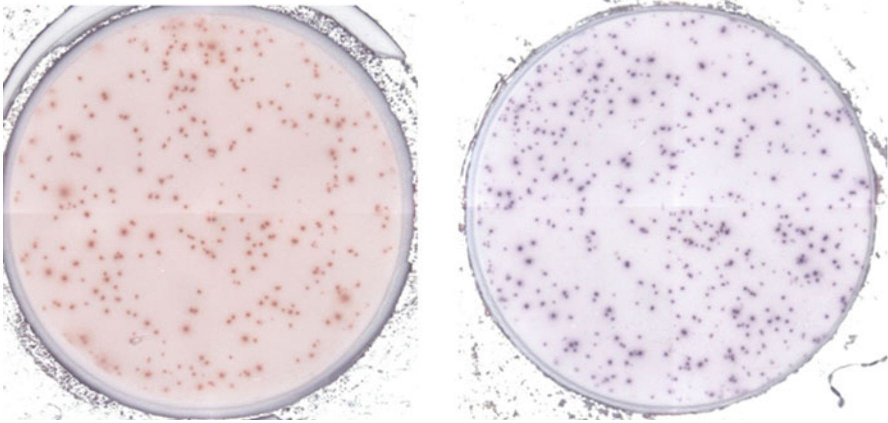

Fig. 2.1 Two wells are depicted from IFNɣ Elispot assays testing human peripheral blood mononuclear cells (PBMCs) for IFNɣ release upon stimulation with the CEF peptide pool. The *blue* and *red* spots were obtained with different enzymatic development systems (reviewed in Sect. 6.9). The membrane bottom of the wells was punched out onto a sealing tape, for optimal visibility of the well periphery. The images were taken with a Zeiss KS Elispot reader system (Thornwood, NY).

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